ANNUAL RESEARCH PROGRESS REPORT

(FY 2004)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

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NUTRITIONAL DETERMINANTS OF HEALTH

MANAGEMENT UNIT

5450-010-00

Project Number: 5450-51000-033-00D **Accession:** 0405041 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 02/22/2002 **Term Date:** 07/20/2004

National Programs: 107 100% Human Nutrition

Title: MINERAL ELEMENT REQUIREMENTS FOR OPTIMAL CARDIOVASCULAR FUNCTION AND HEALTH

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

This project is being replaced by CRIS project # 5450-51000-038-00D, thus, while statement of past accomplishments apply to this terminating CRIS, future milestones are derived from the new OSQR-certified, but as yet unapproved CRIS.

Cardiovascular disease is the leading cause of death in this country, with an annual economic impact projected to approach $180 billion in 2001. Basic research and epidemiological studies have indicated that inadequate dietary intakes of mineral elements such as calcium, copper, magnesium, zinc, as well as overload of iron are associated with altered functions of the heart and circulation. Furthermore, dietary surveys indicate that appreciable numbers of people have sub-optimal intakes of at least some of these minerals. However, we presently have little definitive proof that changing dietary practices with regard to these minerals will benefit cardiovascular health. A clear understanding of how these minerals, particularly at marginal intakes, contribute to cardiovascular function will provide the basis for dietary recommendations that improve the health of the general public. The current project will focus on the contribution of dietary copper to cardiovascular health.

The overall objective is to determine, using animal models, whether copper (Cu) intakes consistent with those observed in humans can adequately support cardiovascular functions. This objective will be pursued through the mechanistic tether of oxidative stress/altered nitric oxide metabolism by which Cu functions, and includes the following specific objectives 1) to develop a strategy for assessment of marginal copper deficiency in animals; to use this strategy to determine biomarkers of copper status that are suitable for assessment of marginal status in humans, 2) to determine the contribution of oxygen- and nitrogen-derived reactive species to the cardiomyopathy (metabolic, contractile) induced by Cu deficiency, and the dietary intakes at which this pathology occurs, 3) to determine whether low Cu intakes consistent with those observed in humans can impair nitric oxide-dependent control of blood vessels and blood pressure regulation, 4) to determine whether the oxidative stress induced by Cu deficiency affects homocysteine metabolism and, thereby, cardiovascular function, and whether such effects influence nitric oxide-dependent signal transduction and/or other mechanisms that affect atherosclerosis, 5) to determine whether marginal Zn deficiency can exacerbate or unmask cardiovascular effects of sub-optimal Cu status by virtue of its role in oxidative/nitrosative metabolism.

Research will address components of National Program 107, Human Nutrition (100%). From component 1, Nutrition Requirements, objectives A (Biomarkers), B (Mechanism of Action), C (Nutrient Interactions), E (Genetic Variability) and G (Function and Performance) will be addressed. From component 2, Diet, Genetics, Lifestyle, and the Prevention of Obesity and Disease, objective A (Identify nutritional,

environmental and genetic factors that modify the effects of nutrient intake and metabolism on health outcomes) will be addressed.

2. **List the milestones (indicators of progress) from your Project Plan.**

Year 1 (FY 2005)

Assess relationships between organ copper and marginal intakes of dietary copper; select organ with best discrimination of copper intake.

Determine if copper deficiency causes reduced mitochondrial respiratory complex activity.

Identify mitochondrial respiratory complexes causing increased hydrogen peroxide production during copper deficiency.

Determine nitric oxide effect on mitochondrial respiration in copper deficiency.

Determine effect of copper deficiency on homocysteine metabolism.

Year 2 (FY 2006)

Determine signaling pathway for induction of inducible nitric oxide synthase during copper deficiency.

Determine nature of effect of altered nitric oxide on blood pressure during copper deficiency.

Determine effect of copper deficiency on bilirubin and biliverdin reductase.

Determine if low zinc acts to exaggerate cardiovascular effects of marginal copper.

Year 3 (FY 2007)

Correlate organ copper content with semi-direct indicators of copper status; select best potential single biomarker(s).

Identify respiratory complexes affected by developmental copper deficiency.

Determine extent of oxidative modification of mitochondrial DNA by copper deficiency.

Determine role of nitric oxide in impaired contractile function of copper deficiency.

Determine relationship between nitric oxide, oxidative stress, homocysteine in atherosclerotic symptoms of copper deficiency.

Clarify role of oxidative/nitrosative stress in zinc/copper interaction.

Year 4 (FY 2008)

Determine nature of effect of altered nitric oxide on coronary vessels during copper deficiency.

Determine role of hemoxygenase in atherosclerotic effects of copper deficiency.

Year 5 (FY 2009)

Correlate organ copper content with combinations of indicators of copper status; select best combination of indicators as biomarker.

Identify oxidized, nitrated mitochondrial proteins in copper deficiency.

Identify mitochondrial DNA mutations of copper deficiency.

Relate mitochondrial DNA mutations to expression of respiratory complexes caused by copper deficiency.

[Determine whether elevation of inducible nitric oxide synthase preconditions copper-deficient hearts (contingency).]

3. **Milestones:**

A. List the milestones that were scheduled to be addressed in FY 2004. How many milestones did you fully or substantially meet in FY 2004 and indicate which ones were not fully or substantially met, briefly explain why not, and your plans to do so.

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[As no formal milestones were in place under the format of this terminating CRIS, the following listing denotes the accomplishments projected for 2004 from the 2003 Annual Report and how each was addressed.]

1) A study will be completed that will determine whether aberrant carbohydrate metabolism (nonenzymatic glycosylation of proteins) causes defects of dietary copper deficiency.

This study has now been completed with negative results and, as it is not a part of the new CRIS plan, will be discontinued.

2) A series of studies will be completed that delineate whether elevated cardiac nitric oxide status plays a role in the altered energy metabolism observed in hearts of copper-deficient rats.

This set of studies is ongoing. Altered energy metabolism with copper deficiency has been established and alterations of nitric oxide signaling have been established in copper-deficient hearts (abstract presented and manuscript in preparation showing elevation of heart nitric oxide synthases), but further technical training will be required for completion of the tests for nitric oxide dependence of mitochondrial respiration.

3) A series of studies will be completed that will determine the role of insulin-like growth factor (IGF-1) and other growth factors in the altered heart function of copper-deficient rats.

A manuscript describing dependence of altered contraction of copper-deficient hearts on IGF-1 is nearing completion as of the writing of this report.

4) A long-term (18-month) study initiated in 2002-03 that is aimed at examining the effect of marginal copper deficiency on parameters related to heart failure (cell death, impaired heart function) in adult rats will be completed.

An abstract has been presented and a manuscript is in preparation describing structural and functional changes in hearts of adult rats fed marginally copper-deficient diets.

5) Groundwork will be laid with commodities producers that is aimed at examining the efficacy of copper-containing foods in ameliorating the defects of dietary copper deficiency in laboratory animals. Studies will be initiated that will determine the appropriate copper-containing dry edible bean to use in copper supplementation trials for animal and human studies.

An animal protocol is being prepared as of the writing of this report.

6) Examination of the effects of maternal copper deficiency on the production of reactive oxygen species by heart mitochondria in neonates will continue. Methodology will be developed to determine if the altered cellular redox state increases oxidative carbonylation and nitration of mitochondrial and extra-mitochondrial proteins in heart tissue and oxidative modifications in the DNA of cardiac mitochondria.

An ELISA has been developed to measure the nitro-tyrosine content in plasma and tissue proteins. This ELISA is ready for application in future studies. An ELISA and an immunoblotting technique using slot-blots are under development and will be ready for application in studies during FY 2005. Literature searches for methods to assess oxidative damage to mitochondrial DNA are underway, but development of the methodology has been delayed because of the extended time involved in developing the methodology for detecting protein nitration and carbonylation.

7) An experiment with animals will be performed that will include the determination

of whether dietary boron influences the beneficial effects of the long-chain omega-3 fatty acids on cardiovascular health.

The animal phase of this experiment has been completed. Completion of assays and data analysis is in progress.

8) A series of studies will be initiated that examine the effect of dietary copper deficiency on homocysteine metabolism in laboratory animals.

Pilot studies of homocysteine analysis were performed on tissues from a study designed for another purpose. Because of methodological difficulties with determination of homocysteine in tissues, this milestone was initiated but not met. Methods are being redeveloped so these experiments can be completed in the future.

9) Low success in recruitment and retention led to insufficient data collection in the human study to determine the excessive dose of zinc supplement at a defined copper intake. The project will continue with improved methods of recruitment.

The human study to determine the excessive dose of zinc supplement at a defined copper intake continues. Recruitment and retention of volunteers has improved. The number of volunteers is slightly less than that suggested by power analysis as being needed. Data are being evaluated to determine whether conclusions can be drawn.

10) Animal experiments will continue to identify new indicators of copper status. This year we will study the enzyme paraoxonase because the literature indicates that it is a leading defender against oxidative stress.

An animal experiment has been performed and a manuscript describing depression of paraoxonase with copper deficiency is under revision. Assay methods for serum lysyl oxidase and tyrosinase are being developed. After validation they will be applied to animal experiments to identify new indicators of copper status.

11) Copper deficiency is the only nutritional insult that produces cardiovascular disease AND bone disease in experimental animals. We will begin experiments with animals to clarify the epidemiologic association between ischemic heart disease and osteoporosis. Dietary copper and measurements of oxidative defense will be prominent.

Mice either susceptible or resistant to atherosclerosis and osteoporosis were supplemented with copper for seven months. Some assays are incomplete; data will be analyzed this year.

12) Chelation therapy of ischemic heart disease is being evaluated in a $30 million trial supported by NIH. We will explore developing a companion grant to evaluate the effect of the treatments on trace element metabolism.

The chelation project was abandoned because we were unable to find suitable medical collaborators at clinics in either Fargo or Grand Forks.

13) A protocol will be developed to test the hypothesis and that copper supplementation will decrease in frequency of idiopathic ventricular premature discharges in patients with otherwise normal heart physiology.

The project on effects of copper supplements on cardiac arrhythmia was abandoned because it was eliminated from the revised CRIS by the OSQR process.

14) As several aspects of the metabolic syndrome resemble findings in people depleted of copper experimentally and in copper deficient animals a protocol will be developed to test the hypothesis that copper supplements will benefit people with this syndrome.

This project was eliminated at the suggestion of the OSQR reviewers.

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15) A study will be completed to identify proteins induced in hearts of copper-deficient rats.

A manuscript is in press that describes induction of two proteins, a procollagen and a DNA-binding protein, in copper-deficient rats.

B. List the milestones that you expect to address over the next 3 years (FY 2005, 2006, & 2007). What do you expect to accomplish, year by year, over the next 3 years under each milestone? [Milestones are from new CRIS Project # 5450-51000-038-00D]

Year 1 (FY 2005)
Assess relationships between organ copper and marginal intakes of dietary copper; select organ with best discrimination of copper intake.

Perform long-term (up to five months) studies of marginal copper deficiency in laboratory animals to determine relative sensitivity of organ copper to marginal copper depletion. Measure cytochrome c oxidase in target organs and platelets and ecSOD in plasma to determine which ones are most sensitive to marginal copper intakes. Measure the inhibitory effects of zinc and iron on the utilization of dietary copper and compare their intensities in rats. Establish white blood cell copper as a measure of copper nutriture in rats. Evaluate serum lysyl oxidase as a marker of marginal copper nutriture in rats. Evaluate tyrosinase as a marker of marginal copper nutriture in rats.

Determine if copper deficiency causes reduced respiratory complex activity.

Measure cytochrome c oxidase and other respiratory complex activities in heart mitochondria from rats fed diets containing deficient, marginal, moderate, and adequate levels of copper.

Identify respiratory complexes causing increased hydrogen peroxide.

Hydrogen peroxide production by heart mitochondria will be measured using either glutamate or succinate as substrate and either in the presence or absence of rotenone or antimycin A to determine maximum rates of hydrogen peroxide generation by complex I and complex III.

Determine nitric oxide effect on mitochondrial respiration in copper deficiency.

Apply selective nitric oxide synthase inhibition to laboratory animals undergoing dietary copper deficiency to determine whether nitric oxide can account for deficit in mitochondrial function caused by copper deficiency.

Determine effect of copper deficiency on homocysteine metabolism.

Methods for determination of tissue homocysteine are being redeveloped. Also, real time RT-PCR assays are currently being developed to study message of enzymes involved with homocysteine metabolism. These assays, along with enzyme analyses will be used to determine the effect of copper deficiency on various aspects of homocysteine metabolism.

Determine whether dry edible beans can supply copper to reverse indices of copper deficiency (pilot to test1).

An animal protocol for this study is currently being developed.

Year 2 (FY 2006)
Determine signaling pathway for induction of inducible nitric oxide synthase.

Examine effects of copper deficiency on protein levels, mRNA, transcription and activation factors for isoforms of nitric oxide synthase in hearts of laboratory animals.

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Determine nature of effect of altered nitric oxide on blood pressure.
Utilize pharmacological inhibition of nitric oxide synthase with L-arginine analogs, in combination with blockade of hormones responsible for blood pressure regulation, to determine the pathways involved in altered blood pressure regulation caused by dietary copper deficiency.

Determine effect of copper deficiency on bilirubin and biliverdin reductase.
The initial finding that bilverdin reductase activity is reduced in copper deficiency will be followed up by additional studies of the relationship between copper status, bilirubin, homocysteine and atherosclerosis.

Determine if low zinc acts to exaggerate cardiovascular effects of marginal copper.
A factorially arranged experiment with rats will be performed to determine whether a moderate zinc deficiency is a stressor of copper metabolism or oxidative metabolism involving copper such that it increases the risk of cardiovascular dysfunction in marginal copper-deficiency.

Year 3 (FY 2007)
Correlate organ copper content with semi-direct indicators of copper status; select best potential single biomarker(s).
Utilizing long-term marginal copper deficiency studies, correlate organ copper content with candidate indirect measures of copper status including cytochrome c oxidase in platelets and extracellular superoxide dismutase in plasma to determine which one are most sensitive to marginal copper intakes. Determine the dietary threshold for coagulation factor V in assessing marginal copper nutriture. Determine the dietary threshold for coagulation factor VIII in assessing marginal copper nutriture. Evaluate diamine oxidase as a marker of marginal copper nutriture in rats. Evaluate the copper chaperone in erythrocytes as a marker of marginal copper nutriture in rats.

Identify respiratory complexes affected by developmental copper deficiency.
Respiratory complex activities and protein subunits of complex I and complex IV will be assayed in the hearts of neonates from marginally Cu-deficient dams at various ages up to one year.

Determine extent of oxidative modification of mitochondrial DNA by copper deficiency.
The content of 8-hydroxydeoxyguanosine will be measured in heart mitochondrial DNA from rats fed diets containing deficient, marginal, and adequate levels of copper.

Determine role of nitric oxide in heart contractile function.
Determine by acute and chronic inhibition of nitric oxide synthase whether nitric oxide plays a role in altered contractile function of hearts from copper-deficient animals.

Determine relationship between nitric oxide, oxidative stress, homocysteine in atherosclerotic symptoms of copper deficiency.
A series of experiments will be initiated that will be aimed at determining the relative effects and interactions between nitric oxide, oxidative stress and homocysteine in causing atherosclerotic vessel damage in marginally copper-deficient animals.

Clarify role of oxidative/nitrosative stress in zinc/copper interaction.
If it is determined that a moderate zinc deficiency exacerbates signs of

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marginal copper deficiency, an experiment with rats will be performed to determine whether cardiovascular effects are modified by changing nitric oxide formation.

4. **What were the most significant accomplishments this past year?**

Copper deficiency during pregnancy and lactation causes genetic effects that affect energy metabolism in the heart.

Cytochrome c oxidase is a copper-dependent enzyme that aids in the use of oxygen by mitochondria, energy-producing structures in cells. Female rats were fed marginally copper-deficient diets during pregnancy and lactation to examine effects on cytochrome c oxidase activity and genetic expression of its subunits in hearts of their offspring. Cytochrome c oxidase activity and expression of two of its subunits, one genetically encoded in the nucleus, the other in the mitochondria, were found to decline 15 days after birth in offspring of copper-deficient mothers. These findings indicate that maternal copper deficiency impairs the activity of cytochrome c oxidase in neonatal heart by not only depriving the enzyme of the copper needed for activity, but also by impairing the genetic expression of its subunits. Because the heart is not capable of rapid repair, the impaired expression of the protein subunits of cytochrome c oxidase may not be easily reversed by adequate dietary copper intake. IMPACT: This and prior studies indicate that long term changes in mitochondrial function related to irreversible changes in cytochrome c oxidase activity could increase cardiac oxidative stress and increase the risk of heart disease in progeny of moderately copper-deficient mothers.

B. Other Significant Accomplishment(s), if any:

Oxidant stress in copper-deficient hearts triggers detrimental cell signaling.

The mitochondrial production of reactive oxygen species (ROS) such as hydrogen peroxide can be increased by partial inhibition of cytochrome c oxidase. The increase in mitochondrial ROS may have two important outcomes: (1) increased oxidative damage to cellular components, and (2) alterations in signaling pathways mediated by reactive oxygen species. We found that inhibition of cytochrome c oxidase by copper-deficiency was related to the induction of heme oxygenase-1 through enhanced production of mitochondrial hydrogen peroxide in liver. Heme oxygenase-1 was also induced in hearts of copper-deficient rats indicating that relationship between copper deficiency, cytochrome c oxidase activity and mitochondrial hydrogen peroxide production may also hold in cardiac tissue. IMPACT: This indicates that cardiac tissue responds to acute/severe copper deficiency by increasing the mitochondrial production of reactive oxygen species that may serve as second messengers in signaling pathways in the heart. Alterations in signaling by mitochondrially generated reactive oxygen species may trigger cell death in cardiac tissue and contribute to the development of heart disease during copper deficiency.

Copper repletion reverses heart effects of copper-deficiency.

Dietary copper deficiency leads to a variety of detrimental changes in the heart, including genetic, structural, and functional changes that are suggestive of heart failure. It is not clear however whether, once initiated, these changes can be reversed. Scientists at the Grand Forks Human Nutrition Research Center, in collaboration with scientists at the University of Louisville, sought to determine whether repletion of copper in the diets of mice fed severely copper-deficient diets from just after birth could ameliorate these changes. Copper repletion, after two or four weeks of severe deficiency, prevented or ameliorated the weight loss of copper deficiency, the detrimental structural changes within heart cells, the impaired contraction and relaxation of the heart and many of the genetic changes observed in copper-deficient hearts. IMPACT: These findings indicate that replenishment of

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copper can avert symptoms of heart failure in severely copper-deficient animals.

Sensitivity to growth factor signals heart failure in copper deficiency.

An anomaly of dietary copper deficiency is that, though overall heart contractile function is depressed, contractility of individual heart cells is enhanced. Heart failure of other causes also shows this elevated contractility as an apparent compensation for reduced function, the apparent cause being an elevation of insulin-like growth factor-I (IGF-I). Scientists at the Grand Forks Human Nutrition Research Center in conjunction with scientists at the University of Wyoming and the University of North Dakota, sought to determine whether an IGF-I mechanism could be implicated in the pathology of copper-deficient hearts. The hearts of copper-deficient rats were found to contain elevated amounts of the receptor for IGF-I and the elevated contractility of heart cells was found to be inhibited by an agent that blocks this receptor. IMPACT: That copper-deficient hearts show apparent compensation via enhanced cellular contractility that is associated with an IGF-I-mediated mechanism provides further evidence of the presence of heart failure in copper-deficient animals.

Copper increases DNA-binding protein in heart.

Dietary copper (Cu) deficiency causes known heart pathology in rodent models. However, alteration in gene expression has not been fully examined. A study was undertaken to determine the effect of Cu deficiency on protein profiles in rat heart tissue. An extract enriched in nuclear protein from heart tissue of copper-deficient rats was found by molecular biology techniques to contain a 132 kDa protein that was not present in Cu-adequate rats. Further analysis revealed that this protein contained a collagen alpha (I) chain precursor as well as a leucine-rich protein 130 (LRP130) and that the protein extract from Cu-deficient rats bound to a single-stranded cytosine-rich DNA with higher affinity than the extract of Cu-adequate rats, similar to reports of an increase in LRP130 single-stranded DNA binding activity in several types of tumor cells. IMPACT: Collectively, these results not only suggest an additional feature of altered collagen metabolism with Cu-deficiency but also demonstrate for the first time an increase in single-stranded cytosine-rich DNA binding in Cu-deficient rat heart.

Boron may enhance cardiovascular effects of fish oil.

Recent findings indicate that dietary boron alters the response of rats to diets with different fatty acid compositions, including the response to diets containing fish oil that reportedly is beneficial to cardiovascular function through reducing circulating cholesterol and modifying prostaglandin formation. At the Grand Forks Human Nutrition Research Center an experiment was performed in which boron-deficient and boron-adequate rats were fed diets with the fat source either fish oil or safflower oil. Compared to safflower oil, fish oil reduced blood cholesterol and a prostaglandin that, when elevated, promotes cardiovascular disease; the reductions were most marked in boron-adequate rats. IMPACT: The findings suggest that consuming foods rich in boron (for example, fruits, vegetables, legumes and nuts) may enhance the beneficial effects of fish oil on cardiovascular function.

Tolerance to copper in drinking water is high.

Regulatory agencies place limits on concentrations of chemicals in drinking water. In the case of copper, the limit is based on nausea threshold. In an international, multi-center trial that included the Grand Forks Human Nutrition Center, copper solutions in commercial mineral water were provided to fasting women. Copper up to 4 mg per liter was well-tolerated. IMPACT: This experiment validates the EPA and WHO standards of 1.3 and 2.0 mg copper per liter of drinking water, respectively, and will be helpful in planning clinical trials of copper supplements.

Antioxidant enzyme is depressed in copper deficiency.

Paraoxonase is an enzyme that protects against oxidative damage; low activity also predicts heart disease risk in people. Rats were fed a diet severely deficient in copper to test the hypothesis that paraoxonase activity would be decreased as is well-known for other anti-oxidant functions of copper. Paraoxonase was found to have decreased activity in rats deficient in copper. IMPACT: This finding suggests the possibility of a new biomarker for severe copper deficiency.

C. Significant activities that support special target populations.
None.

D. Progress Report opportunity to submit additional programmatic information to your Area Office and NPS (optional for all in-house ("D") projects and the projects listed in Appendix A; mandatory for all other subordinate projects).
None.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Magnesium prevents heart arrhythmia.

Physiological evidence of a requirement for magnesium by healthy persons has not previously been shown. Postmenopausal women subjects at the Grand Forks Human Nutrition Research Center were fed diets that contained 130 mg of magnesium daily or an amount (411 mg daily) greater than the recommended dietary allowance for six months, during which electrocardiographic measurements were made. Subjects consuming the lower amount of dietary magnesium had lower magnesium in red blood cells and in blood serum and a greater number of increased abnormal heart beats compared to those consuming the higher amount of magnesium. IMPACT: These findings illustrate by use of physiological criteria that the magnesium requirement for postmenopausal women exceeds 130 mg daily. NP 107 Action Plan Component 1, Objective G.

Copper deficiency causes signs of heart failure.

Although many contractile, electrical and metabolic abnormalities have been found in hearts of copper-deficient animals, characteristics that unequivocally indicate heart failure have not been demonstrated. Scientists at the Grand Forks Human Nutrition Research Center, in collaboration with scientists at the University of Louisville, measured variables in hearts of copper-deficient mice that are used in humans to demonstrate heart failure. Hearts of copper-deficient mice were found to have reduced maximum contractile pressure, elevated pressure during relaxation, reduced responsiveness to excitation by adrenalin and increased collagen deposits, all of which are signs of heart failure. IMPACT: This suggests that dietary copper deficiency is a risk factor for heart disease and subsequent cardiac failure. NP 107 Action Plan Component 1, Objectives B and G.

Impaired blood pressure regulation in copper deficiency is related to nitric oxide.

Although prior studies have shown that blood vessels of copper-deficient rats respond poorly to agents that stimulate release of nitric oxide, a potent endogenous dilator, it has not been shown that this causes an alteration of blood pressure. In studies at the Grand Forks Human Nutrition Research Center, rats were injected with a drug (L-NAME) that inhibits formation of nitric oxide and elevates blood pressure. The elevation of blood pressure by L-NAME injection was found to be depressed in rats that were fed inadequate copper. IMPACT: This indicates that dietary copper deficiency causes an impairment of blood pressure regulation and a predisposition to high blood pressure. NP 107 Action Plan Component 1, Objectives B and G.

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Copper deficiency enhances atherosclerosis-like blood vessel damage.

Although dietary copper deficiency has been shown to enhance inflammation, the relationship between dietary copper and blood vessel injury has not been previously examined. The Grand Forks Human Nutrition research Center, in collaboration with Drs. D.A. Schuschke and J.J. Dalle Lucca at the University of Louisville, examined effects of injury induced by balloon inflation within blood vessels of copper-deficient and copper-adequate rats. Blood vessel injury by balloon inflation within the vessel caused thickening of the blood vessel wall (akin to atherosclerosis) that was exaggerated by restriction of dietary copper. IMPACT: This finding provides further support for the view that the inflammatory response to injury is exaggerated by copper deficiency and, further, because balloon inflation of blood vessels (angioplasty) is a common mode of treatment for atherosclerotic blood vessels, the findings suggest that proper copper nutrition may improve the results of such treatment. NP 107 Action Plan Component 1, Objectives B and G.

Copper deficiency during pregnancy causes long-term effects in offspring.

Women of child-bearing age consume less than the recommended daily requirement of copper, but it is not known if low dietary copper intake during pregnancy has long-term effects on the cardiovascular system of children. To test this possibility in laboratory animals, scientists at the Grand Forks Human Nutrition Research Center bred rats to bear and nurse offspring during maternal copper deficiency; pups were then fed copper-adequate diets for nine months following weaning and assessed for altered heart mitochondrial function, enzymes and oxidative stress. Activities of respiratory complexes of heart mitochondria in neonates from copper deficient dams was significantly reduced at 21 days following birth and could not be restored to normal activity levels by six weeks of copper supplementation. Further, despite nine months of copper repletion, rats of copper-deficient dams exhibited an abnormally low activity of cytochrome c oxidase, a copper-dependent enzyme, in heart mitochondria and an increase in heart mitochondrial hydrogen peroxide production. IMPACT: This finding indicates that copper deficiency during pregnancy has long-term, possibly irreversible, effects on energy metabolism in the hearts of neonates that could increase their susceptibility to heart disease as adults. NP 107 Action Plan Component 1, Objectives B and G.

Dietary magnesium and nickel interact to benefit cardiovascular health.

In vitro and toxicity studies indicate that responses to dietary nickel can be altered by magnesium status. In an experiment performed at the Grand Forks Human Nutrition Research Center, urine was collected at 8 and 12 weeks and blood at 13 weeks after weanling rats were placed on diets containing marginally deficient or adequate magnesium, and containing low or physiologically normal amounts of nickel, for the determination of variables associated with cardiovascular health. Low dietary nickel increased the urinary excretion of prostaglandin E-2 and sodium, and the plasma concentrations of triglycerides and glucose; marginal magnesium deficiency enhanced the changes in urinary variables. IMPACT: The findings suggest that nickel and magnesium interact at the kidney level in a manner that can affect blood pressure, that low dietary nickel causes lipid changes undesirable for heart health, and that consuming foods such as leafy green vegetables, legumes, whole grains and nuts, is beneficial to cardiovascular health partly because they provide goodly amounts of nickel and magnesium. NP 107 Action Plan Component 1, Objectives B, C and G.

Zinc deficiency exaggerates reduced magnesium balance in postmenopausal women.

Showed that a moderately high intake of zinc (53 mg per day) compared to a deficient intake of zinc (3 mg per day) decreased magnesium balance in post-menopausal women apparently by decreasing the amount of magnesium absorbed from the

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diet. IMPACT: The finding suggests that individuals consuming less than recommended amounts of magnesium may have deficiency signs, including changes in risk factors associated with cardiovascular dysfunction , exacerbated by taking supplements providing high amounts of zinc. NP 107 Action Plan Component 1, Objectives B, C and G.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**

The usual routine transfer of nutritional knowledge about the nutritional, beneficial, and non-beneficial effects of trace elements was made through direct contact with industry representatives and the public and with other scientists through presentations at national and international meetings and professional publications.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**

W.T. Johnson wrote an article which appeared on March 10, 2004 in the nutrition section of the Grand Forks Herald titled "Come along on a search for a good carbohydrate."

E.O. Uthus wrote an article entitled "Folic acid: For women and men," which appeared in the Grand Forks Herald on July 7, 2004.

L.M. Klevay presented a lecture entitled "Scientific Myths: Old and New" at the Rotary Club, Grand Forks, North Dakota.

L.M. Klevay presented a lecture entitled "Copper Nutriture and Ischemic Heart Disease," at the University of Texas Medical Branch, Department of Preventive Medicine and Community Health, Galveston, TX.

L.M. Klevay presented a lecture entitled "Ischemic Heart Disease as Copper Deficiency" at the University of Washington, Center for Public Health Nutrition, Seattle, WA.

L.M. Klevay presented a lecture entitled "The Copper Deficiency Theory of Ischemic Heart Disease" at the University of Southampton, Department of Clinical Epidemiology, Southampton, UK.

L.M. Klevay presented a lecture entitled "Copper Deficiency and Cardiovascular Disease in Humans" at the University of Surrey, School of Biomedical and Molecular Sciences, Guildford, UK.

L.M. Klevay answered several inquiries from individual citizens about copper in the United States diet, recommended dietary allowances, best chemical form for copper supplements, treatment of copper deficiency and anti-copper drugs.

Cardiovascular research by L.M. Klevay was featured in an article, entitled "Lack of Energy? Maybe it's your Magnesium Level" by Rosalie M. Bliss. In Agricultural Research, May 2004, p. 8-9.

Scientific Publications: **Log 115:**

1. Araya, M., Chen, B., Klevay, L.M., Strain, J.J., Johnson, L., Robson, P., Shi, W., Nielsen, F., Zhu, H., Olivares, M., Pizarro, F., Haber, L.T. 2003. Confirmation of an acute no-observed-adverse-effect and low-observed-adverse-effect level for copper in bottled drinking water in a multi-site international study. Regulatory Toxicology and Pharmacology. 38:389-399. 0000155186

2. Elsherif, L., Wang, L., Saari, J.T., Kang, Y.J. 2004. Regression of dietary copper restriction-induced cardiomyopathy by copper repletion in mice. Journal of Nutrition. 134:855-860. 0000161756

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3. Elsherif, L., Jiang, Y., Saari, J.T., Kang, Y.J. 2004. Dietary copper restriction-induced changes in myocardial gene expression and the effect of copper repletion. Experimental Biology and Medicine. 229:616-662. 0000164116

4. Johnson, W.T., Demars, L.C. 2004. Increased heme oxygenase-1 expression during copper deficiency in rats results from increased mitochondrial generation of hydrogen peroxide. Journal of Nutrition. 134:1328-1333. 0000159639

5. Lominadze, D., Saari, J.T., Percival, S.S., Schuschke, D.A. 2004. Proinflammatory effects of copper deficiency on neutrophils and lung endothelial cells. Immunology and Cell Biology. 82:231-238. 0000156835

6. Zhou, Z., Wang, L., Song, Z., Saari, J.T., McClain, D.J., Kang, Y.J. 2004. Abrogation of nuclear factor-kappa-B activation is involved in zinc inhibition of lipopolysaccharide-induced tumor necrosis factor-alpha production and liver injury. American Journal of Pathology. 164(5):1547-1556. 0000161726

7. Nielsen, F.H. 2003. Trace elements. Book Chapter. In: Katz, S.H., Weaver, W.W., editors. Encyclopedia of Food and Culture. New York, NY: Charles Scribner's Sons. p. 410-415. 0000133381

8. Klevay, L.M. 2004. Chemical validation of X-ray absorptiometry [Letter to Editor]. American Journal of Clinical Nutrition. 79:887. 0000156231

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14. Klevay, L.M. 2004. Copper deficiency decreases paraoxonase activity in rats [abstract]. Federation of American Societies for Experimental Biology Journal. 18:A915. 0000156506

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Approved: ROOS ERIC E **Date:** 09/14/2004

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 02/22/2002 **Term Date:** 09/30/2004

National Programs: 107 100% Human Nutrition

Title: MINERAL INTAKES FOR OPTIMAL BONE AND JOINT DEVELOPMENT AND HEALTH

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

Osteoporosis is a prevalent and costly bone disease that causes at least one bone fracture in one out of every two women and one out of eight men in their lifetimes. The annual cost of healthcare related to osteoporosis is estimated at $14 billion. In the elderly, hip fractures are associated with mortality in up to 20% of the cases. Osteoporosis is a multifactorial disease process directed by several factors. These risk factors include environment (i.e., nutrition, physical activity), genetics, endogenous hormones (estrogen and androgen deficiencies), and age. Behavior modification and pharmacologic intervention (e.g., hormone replacement therapy [HRT]) are the common complementary approaches used to prevent bone loss in asymptomatic women. However, only 12-20% of US postmenopausal women currently use HRT. Thus, nutrition is, arguably, one of the most important modifiable factors and represents a primary approach in prevention of this debilitating disease. For this reason, all significant nutritional factors must be identified that maximize peak bone mass during development and maintain bone mass and strength during aging.

There is consensus that adequate calcium intake continuous from childhood is critical for the formation and maintenance of a healthy skeleton. Thus, dietary calcium supplementation is considered as both a treatment and prophylaxis for osteoporosis. However, there is no current recommended daily allowance (RDA) for this important nutrient because experimental data are not available to assess adequately the physiological adaptation to changes in calcium intake over time. Furthermore, calcium metabolism is known to be modified by many other dietary factors including dietary protein sources (vegetable and animal), trace minerals (e.g., copper, zinc, and boron), and prebiotics (e.g., inulin). However, to date, the interaction of these factors with calcium is at best ill-defined. Therefore, this project has two specific goals: 1) titration of the calcium intake needed to optimize bone health and prevent bone loss in postmenopausal women and 2) determination of the roles of key dietary factors (protein, selected trace elements, and prebiotics) on the utilization of dietary calcium.

Animal and human experiments are and will be conducted with the goal of establishing the calcium requirement and characterizing the modifying roles of inulin (a prebiotic), animal proteins (in meat and milk), and zinc, copper, and boron (as trace elements) on that requirement. Postmenopausal women and appropriate animal models (e.g., ovariectomized female rats) consumed varying amounts (low, adequate, and/or supranutritional) of calcium and the identified dietary factors while all other components of the diet remained constant. The response of the animals and humans to the dietary manipulations was ascertained by evaluating appropriate biochemical, physiological, and anatomical variables.

The research to be undertaken falls under National Program 107, Human Nutrition,

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2004

and addresses goal 3.1.1 (Human Nutrition Requirements). The challenge of this component is to identify essential nutrients, determine their effects on reproduction, development, function and longevity, and to provide information that will be used to develop standards to optimize human health, well-being, and genetic potential throughout the life cycle. All priority objectives, especially mechanism of action, biomarkers, function and performance, and nutrient interactions apply to the research program. Outcomes of the research will be knowledge that will facilitate establishment of an RDA for calcium with due consideration of the dietary factors that have significant potential to modify that requirement.

2. **List the milestones (indicators of progress) from your Project Plan.**
Because this project plan is under development, no formal milestones are in place. The Project Plan for FY 2004 -9 recently completed an ad hoc review (Composite Action Class: Major Revision) and is being revised.

3. **Milestones:**
A. Research activities for FY 2004.

Studies planned for and executed in FY 2004 are listed below:

Study 1. The hypothesis that silicon is an essential nutrient involved in the formation or function of bone matrix components including collagen and glycosaminoglycans which, if suboptimal, increases the risk for osteoporosis will be tested by using a SASo-2 cell culture system to ascertain the effect of silicon at the molecular level. OUTCOME: A SASO-2 cell culture system was established in an attempt to determine the role of silicon in bone matrix formation.

Study 2. An experiment will be performed with experimental animals to determine whether urinary and blood markers of bone turnover are altered by relationship between boron and the omega-3 fatty acids that could be used as a basis for a human study to ascertain the influence of these dietary substances on the susceptibility or prevention of osteoporosis. OUTCOME: The animal portion of the study was completed and biochemical and bone morphological determinations are partially completed.

Study 3. An experiment will be performed in collaboration with the University of Buenos Aries to determine whether silicon status affects the formation of bone around dental and bone implants. OUTCOME: The animal portion of the study was completed and biochemical and bone histological determinations are partially completed.

Study 4. An experimented will be completed that determines whether dietary silicon helps prevent bone loss in an animal model of osteoporosis (ovarioectomized adult rats). OUTCOME: Study was completed.

Study 5. The previously initiated three-year, double-blind placebo-controlled supplementation trial will continue to compare the effects of calcium supplementation with and without copper and zinc supplementation on bone loss in postmenopausal women. OUTCOME: Recruitment and data collection for this previously initiated study continues. A total of 220 women are needed. Currently, we have successfully recruited 179 women into the study.

Study 6. Controlled feeding studies designed to test the effects of interaction between meat protein and dietary calcium on calcium retention and bone metabolism will be conducted. OUTCOME: The feeding phase of this study is complete and sample analysis is in progress.

Study 7. Animal studies investigating the interaction of meat protein and dietary calcium on calcium retention and biomechanical and biochemical properties of bone as well as the IGF-1 proteins will be conducted in ovarioectomized rats. OUTCOME: This study has been completed.

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Study 8. Studies will be initiated to test the feasibility of use of calcium-41 combined with accelerated mass spectrometry in nutrition research, with emphasis on bone metabolism. OUTCOME: A pilot study was completed, in collaboration with the Lawrence Livermore National Laboratory, using archived human urine samples from a previous study. The results, which were presented this year in a workshop held in Zurich Switzerland and at the Experimental Biology 2004 meeting in Washington, DC, indicate that we can successfully measure Ca-41 in the human samples in very small quantities using accelerated mass spectrometry.

Study 9. Controlled feeding studies designed to compare calcium retention from soy milk versus dairy milk will be conducted. OUTCOME: This study was not conducted because a grant application for support from the National Dairy Council was not funded.

Study 10. Because boron in the diets has many beneficial effects on human and animal physiology, an experiment will be designed and initiated to examine the uptake and retention of boron from a common food source in healthy volunteers. OUTCOME: In preparation for a human study, broccoli (a good source of dietary boron) samples were analyzed for boron content to determine whether boron content is affected by geographical location, fertilizer method (conventional versus organic), organic fertilizer source (fish meal, phytamin, compost, or guano) and water stress (80, 100, and 150% of water requirement). The samples were provided as part of a collaboration with Dr. John Finley who designed the broccoli production experiment.

Study 11. Boron deprivation is known to affect inflammation in humans and animal models. A study initiated previously will be continued to determine the effects of boron deprivation on manifestation of rheumatoid arthritis in human subjects with an emphasis on measuring variables that will indicate whether low boron nutriture, not uncommon in the United States, is a factor in the prevalence and severity of rheumatoid arthritis. OUTCOME: Per development of the Project Plan, this study was not continued.

Study 12-13. Studies will be initiated to elucidate the induction mechanism whereby boron increases the concentration of natural killer cells in the serum of animal models with experimental rheumatoid arthritis. Animal studies will be conducted to determine why a semi-purified diet based on ground corn and supplemental with boron protect better against experimental arthritis than does a commercial rodent chow with ample boron. OUTCOME: Per development of the Project Plan, a series of alternate studies were designed in collaboration with Dr. David Bradley at the University of North Dakota to determine whether physiological levels of boron prevent or ameliorate collagen-induced arthritis, an animal model of polyarthritis similar to human rheumatoid arthritis (RA). The studies were completed.

Study 14. Boron analysis of human milk samples from mothers living in Houston, TX, were analyzed for boron content to confirm previous findings from this laboratory that the boron content of milk remains constant through the first 4 months of lactation.

Study 15. An animal study was designed and completed to extend our previous finding that boron affects plasma insulin levels during conditions of vitamin D deficiency.

Study 16. An animal study was designed and is in progress to determine the effect of consuming a boron low in on plasma insulin concentrations in those animals that have an impaired glucose tolerance response early in life.

B. Research Plans for FY 2005, 2006, and 2007

FY 2005:

Continue recruitment and data collection for the previously initiated three-year, double-blind placebo-controlled supplementation trial designed to compare the

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2004

effects of calcium supplementation with and without copper and zinc supplementation on bone loss in postmenopausal women.

Complete sample and statistical analyses and report the results for the recently conducted controlled feeding study designed to test the effects of interaction between meat protein and dietary calcium on calcium retention and bone metabolism.

Continue method development for the use of Ca-41 and accelerated mass spectrometry in determination of bone resorption in human subjects.

Conduct controlled feeding studies, using calcium-41/accelerated mass spectrometry methodology, to test the effects of high protein weight loss diets on calcium retention and bone health in postmenopausal women.

Conduct animal studies to determine the effects and mechanisms of action of interaction of trace minerals with calcium on the IGF-1 proteins, calcium retention and bone status in growing and ovarioectomized rats.

Conduct studies with growing and ovariectomized mature rats to determine whether lifelong dietary boron deprivation will exacerbate the decline in tibial trabecular plate density and bone strength during postmenopausal osteoporosis.

FY 2006:

Conduct controlled feeding study to determine the effects of non-digestible carbohydrates, specifically inulin, on calcium retention in postmenopausal women.

Complete sample and statistical analyses and report the results for the recently conducted controlled feeding study designed to test the effects of high protein weight loss diets on calcium retention and bone health.

Conduct animal studies to determine whether uncomplicated boron deficiency retards maturation of the epiphysial plate proliferative zone and induces distortion of the marrow sprouts.

Conduct animal studies to determine whether dietary boron improves calcium absorption especially when calcium intakes are marginal.

FY 2007:

Conduct controlled feeding study to determine the effects of isolated milk proteins versus meat protein on calcium retention will be conducted.

Complete sample and statistical analyses and report the results for the recently conducted controlled feeding study designed to test to determine the effects of non-digestible carbohydrates on calcium retention.

Conduct controlled feeding studies, using calcium-41/accelerated mass spectrometry, to test the effects of graded intakes of calcium on bone resorption with the goal of defining calcium requirements in postmenopausal women.

Conduct animal studies to determine whether uncomplicated boron deprivation in utero will affect soft tissue structure of developing long bones and/or peak bone mass during adolescence.

4. **What were the most significant accomplishments this past year?**

A. Single Most Significant Accomplishment during FY 2003:

To determine whether animal protein intake is a risk for the development of osteoporosis, a carefully controlled feeding study of several weeks duration was conducted in postmenopausal women at the Grand Forks Human Nutrition Research Center. Findings from this study indicate that a high protein diet (20% of energy mostly provided as meat) may not increase calcium loss and or adversely affect bone health in healthy postmenopausal women. On the contrary, a significant improvement in calcium retention was observed in women with low calcium intake. Impact: These findings, which contradict prevalent assumptions about the effects of meat consumption on calcium retention, will improve the quality and nature of the advice

given to the public regarding the effects of dietary protein, especially meat protein, on bone health.

B. Other Significant Accomplishment(s):

A study to determine the effects of high-isoflavone soy protein versus beef on calcium retention and biomechanical properties of bones was conducted at the Grand Forks Human Nutrition Research Center in collaboration with Dr. Wagner at the University of North Dakota. In this study with ovarioectomized rats (estrogen-deficient), the findings indicated that calcium retention was significantly improved when protein intake was high (20%) and it was higher in the animals consuming beef versus soy protein. Serum IGF-1, a growth factor needed for bone health, was also higher in the animals consuming the high protein diets (20% soy or beef). Impact: These results are consistent with our findings from human studies and indicate that a high protein diet may be protective against bone loss in postmenopausal women rather than harmful.

Studies were conducted with rats to determine whether silicon affects collagen formation at the Grand Forks Human Nutrition Research Center in collaboration with Drs. Carol Seaborn and Scott Zimmerman at the University of Wisconsin, Stout. Silicon deprivation increased activity of a gene associated with type I collagen synthesis. Impact: The changes suggest that dietary silicon may have a role in collagen synthesis.

An experiment was performed at the Grand Forks Human Nutrition Research Center to determine whether silicon deficiency in mature rats would exacerbate bone loss induced by ovariectomy (a model of osteoporosis). Silicon deprivation did not affect calcified bone changes induced by ovariectomy, but independently changed blood markers of bone metabolism and bone composition associated with the organic matrix. Impact: The findings suggest that the effects of ovariectomy are mostly on the calcified portion of bone, while the effects of silicon are mostly on the organic matrix.

To characterize further boron concentrations in human milk, a study was conducted at the Grand Forks Human Nutrition Research Center in collaboration with Dr. Nancy Butte (Baylor College of Medicine, Houston, TX). Similar to the findings of a previous study conducted at the Center with milk samples from St. John's, Newfoundland, the average concentration of boron in milk from nursing mothers living in Houston, TX, is 0.03 milligrams per liter and, similar to calcium, remains stable during the first 12 weeks of lactation. Impact: These findings extend the available data on boron contents of human milk making it possible to impute boron intakes of nursing infants.

Studies were conducted at the Grand Forks Human Nutrition Research Center in collaboration with Dr. David Bradley (University of North Dakota) to characterize further the effect of dietary boron on the onset of collagen-induced arthritis (an animal model of polyarthritis similar to human rheumatoid arthritis). Similar to our previous studies, mice weaned onto low-, adequate-, or luxuriant-boron diets exhibited a 54, 29, and 13% incidence of arthritis respectively. Impact: The findings indicate that dietary boron provides resistance to clinical signs in this animal model of rheumatoid arthritis.

C. Other significant accomplishments(s), if any.

None

D. Progress Report opportunity to submit additional programmatic information to your Area Office and NPS (optional for all in-house ("D") projects and the projects listed in Appendix A; mandatory for all other subordinate projects.

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2004

None

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

A study was conducted with growing female rats to determine whether an interaction between calcium and iron affects the biomechanical and biochemical properties of bone. The findings from this study, done in collaboration with Dr. John Wagner (University of North Dakota), indicate that higher than adequate calcium intakes reduced both iron status and bone quality. Impact: These findings indicate that very high calcium intakes, as may result from indiscriminate supplementation and fortification of foods with calcium, may compromise the iron status of vulnerable segments of the population without offering additional benefits to bone health.

Studies were conducted with rats to determine whether dietary silicon affects collagen turnover in bone. Low dietary silicon increased the urinary excretion of two bone-collagen breakdown products and a plasma protein involved in bone turnover. Impact: The changes in these products indicate dietary silicon may have a role in bone and joint collagen turnover.

A study was conducted to determine whether bone health is impaired by a combined lack of dietary nickel and magnesium, two minerals generally supplied by the same foods. The combined marginal deficiency of magnesium and nickel resulted in increased prostaglandin E-2 excretion and changed the mineral composition of bone. Impact: The findings indicate that consuming foods high in both magnesium and nickel, including leafy green vegetables, legumes, whole grains and nuts, is beneficial to bone health, and that the beneficial effects possibly occur through these minerals influencing the metabolism of lipids to prostaglandins.

A high meat intake is often cited as a risk factor for development of osteoporosis, and soy protein is thought to have a beneficial effect on bone health. However, new reported findings indicate that partial substitution of soy protein (with high isoflavones) for meat protein does not improve calcium retention in postmenopausal women. In a follow-up study with ovarioectomized rats, complete substitution of meat protein by soy protein isolate with high isoflavone content reduced calcium retention by about 30%. Impact: Assuming that the findings in rats are applicable to humans, these combined results indicate that, contrary to popular belief, meat protein intake does not adversely affect bones and that while a partial substitution of soy protein for meat protein may not reduce calcium retention, full replacement may adversely affect calcium retention.

We collaborated with Dr. James Friel at the University of Manitoba, Canada, to determine the concentration of boron in human milk from mothers of full-term and premature infants living in St. John's, Newfoundland, Canada. The findings indicate that the average concentration of boron in milk from nursing mothers from this locale is 0.03 milligrams per liter, is not affected by infant prematurity and, similar to calcium, remains stable during the first 12 weeks of lactation. Impact: The findings provide the first data available to impute boron intakes of nursing infants.

The US Food and Drug Administration has approved a health claim that a daily consumption of 25 g of soy protein is beneficial to heart health. However, the effects of this dietary practice on bone health are unknown. A controlled feeding trial with postmenopausal women, using sensitive whole body counting methodology, demonstrated that when 25 g of soy protein is substituted for an equal weight of meat protein, on a daily basis for 8 weeks, the amount of calcium that the body retains does not change. Furthermore, urinary and blood biomarkers of bone formation and breakdown did not change. Impact: These findings indicate that the

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2004

common practice of substitution of soy protein for meat protein does not provide any additional benefits (associated with phytoestrogens) or risks (associated with phytate) with regards to calcium retention or bone health in postmenopausal women and that the effects of vegetable proteins versus animal proteins on bone health deserve further investigations.

We conducted two separate studies with healthy rats and a third with chicks to determine whether boron, a natural substance in the diet, helps regulate the level of insulin in the blood. Results from the rat studies demonstrated that a diet with normal physiological amounts of boron reduced blood insulin levels without affecting glucose levels. In chicks given glucose through a vein injected, boron decreased peak pancreatic insulin release. Impact: These results suggest that dietary boron, found in higher concentrations in fruits, vegetables, nuts, and legumes, may help reduce the amount of insulin needed to maintain blood glucose.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
Information about the nutritional of beneficial aspects of ultratrace and trace elements as it became available was routinely transferred to a variety of customers. The customers included nutritional risk assessment groups through direct contact or organized meetings and workshops; the public through web pages of professional organizations, via the popular media; and other scientists through presentations at national and international meetings and professional publications.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
Information was transferred to the public through the local newspaper (Grand Forks Herald) that was also placed on the Grand Forks Human Nutrition Research Center Home Page. Dr. Roughead wrote two articles entitled, "Protein and calcium: Friends or Foes?" and "Vitamin D: It's good for your bones." Dr. Hunt wrote an article entitled, "Metabolic Syndrome: You may already have it."

Dr. Roughead provided information regarding nutrition and bone health in a North Dakota Public Radio broadcast entitled, "Hear It Now."

Dr. Roughead's recent findings regarding the effects of dietary protein and the role of acid-base balance on bone health were featured in the LA Times and Vogue magazines.

Roughead, Z.K. 2004. Trace Minerals and Bone Health. Presentation given at the Optimist Club, March 17, 2004. Grand Forks, ND.

Roughead, Z.K. 2004. Nutrition for Bone Health. Presentation given at the Lions Club. April 7, 2004, Grand Forks, ND.

Scientific Publications: **Log 115:**

1. Bakken, N.A., Hunt C.D. 2003. Dietary Boron Decreases Peak Pancreatic In Situ Insulin Release in Chicks and Plasma Insulin Concentrations in Rats Regardless of Vitamin D or Magnesium Status. Journal of Nutrition. 133: 3577-3583. **0000144822**
2. Nielsen, F.H., Milne, D.B. 2004. A moderately high intake compared to a low intake of zinc depresses magnesium balance and alters indices of bone turnover in postmenopausal women. European Journal of Clinical Nutrition. 58:703-710. **0000145203**

Project Number: 5450-51000-034-00D **Accession:** 0405069 **FY:** 2004

3. Ralston, N.V., Hunt, C. 2004. Transmembrane partitioning on boron and other elements in RAW 264.7 and HL60 cell cultures. Biological Trace Element Research.98(2):181-191. 0000143122

4. Hunt, C.D. 2003. Dietary Boron: An Overview of the Evidence for its Role in Immune Function. Journal of Trace Elements in Experimental Medicine. 16:291-306. 0000147338

5. Roughead, Z.K. 2004. Dietary protein and calcium: friends or foes? Invited Commentary. Soy Connection 12:4-8 in Journal of American Dietetic Association. 104(7). 0000163669

6. Roughead, Z.K., Hunt, J.R. 2003. Letter to Editor. Reply to Sebastian and Remer and Manz. Journal of Nutrition. 133:3240. 0000151813

7. Hillegonds, D.J., Roughead, Z.K., Vogel, J.S. 2004. Ultra low dose 41ca: testing the detection limits of accelerator mass spectrometry-based analysis in nutrition research [abstract]. Federation of American Societies for Experimental Biology Journal. 18:A925. 0000156305

8. Hunt, C.D., Friel, J. 2004. Boron concentrations in milk from mothers of full-term, but not premature, breast-fed infants remain stable during the first three months of lactation while copper, iron, selenium, and zinc concentrations decrease regardless of gestational length [abstract]. The Federation of American Societies for Experimental Biology Journal. 18:A492. 0000156262

9. Hull, S.C., Burrack, A., Ek-Pangerl, K.E., Nardini, D.M., Hopps, J.L., Seaborn, C., Zimmerman, S.D., Nielsen, F.H. 2004. Low dietary silicon increases tnf-a and collagen gene promoter activity in mice [abstract]. Federation of American Societies for Experimental Biology Journal. 18:A527. 0000157052

10. Nielsen, F.H., Stoecker, B. 2004. Nutritional intakes of silicon affect vertebral trabecular microarchitecture and strength, but not femoral or vertebral strength changes induced by ovariectomy, in rats [abstract]. Federation of American Societies for Experimental Biology Journal. 18:A919. 0000156267

11. Roughead, Z.K., Johnson, L.K., Wagner, J.L. 2003. Dietary iron and calcium interact to affect iron status and bone health in a response surface study of growing female rats [abstract]. Journal of Bone and Mineral Research. 16:S155. 0000149022

12. Roughead, Z.K., Hunt, J.R., Lykken, G.I., Johnson, L.K. 2003. Effects of daily substitution of soy protein for meat protein on calcium retention and biomarkers of bone and cardiovascular health in postmenopausal women [abstract]. Presented at the 5th International Symposium on the Role of Soy in Preventing and Treating Chronic Disease, Orlando, FL, September 21 - 25 , 2003. 0000150851

13. Beuchler, J.L., Roughead, Z.K. 2004. Do meat and calcium interact to affect calcium retention and bone density in ovarioectomized rats? Poster presentation at the Frank Low Research Day, School of Medicine and Health Sciences, University of North Dakota. April 15, 2004. 0000163875

14. Roughead, Z.K. 2004. Radiotracer and whole body scintillation counting methodology: the gold standard for mineral bioavailability studies [abstract]. Presentation given at the Research Day held at Missouri University, Columbia, MO, May 12, 2004. (Invited) 0000164814

Approved: ROOS ERIC E **Date:** 09/14/2004

Project Number: 5450-51000-034-02T **Accession:** 0407132 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 02/01/2003 **Term Date:** 01/31/2006

National Programs: 107 100% Human Nutrition

Title: MEAT PROTEIN AND CALCIUM: DO THEY INTERACT SYNERGISTICALLY OR ANTAGONISTICALLY?

Period Covered From: 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0410

Organization Name: NATIONAL CATTLEMEN'S BEEF ASSOCIATION

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
   A. None
   B. None
   C. None
   D. This report serves to document research conducted under trust agreement #58-5450-3-410 between ARS and the National Cattleman's Beef Association. Additional details of this project can be found in the report for parent CRIS 5450-510000-034-00D.

   To better define the relationship between dietary calcium and meat protein, we have successfully conducted a carefully controlled feeding study in 27 postmenopausal women. Data analysis is in progress. The preliminary findings are summarized in the parent CRIS and will be reported at the 2004 Annual meeting of The American Society for Bone and Mineral Research in Seattle, Washington.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51000-034-04T **Accession:** 0407514 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 10/01/2001 **Term Date:** 09/30/2006

National Programs: 107 100% Human Nutrition

Title: EFFECTS OF PROTEIN ON CALCIUM RETENTION AND BONE METABOLISM IN POSTMENOPAUSAL WOMEN

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-2-0401

Organization Name: NORTH DAKOTA BEEF COMMISSION

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
   A. None
   B. None
   C. None
   D. This report serves to document research conducted under trust agreement #58-5450-2-0401 between ARS and the North Dakota Beef Commission. Additional details of this project can be found in the report for parent CRIS 5450-510000-034-00D.

   During the life of this project we have found that when healthy postmenopausal women consume 25 g of soy protein in place of an equivalent amount of meat protein for several weeks, calcium retention and measures of bone and cardiovascular health are not affected. This is a significant finding as animal protein is often cited as a risk factor for debilitating chronic diseases such as osteoporosis and heart disease. A manuscript summarizing the results has been provisionally accepted for publication in the J. Clinical Endocrinology and Metabolism.

   As a follow-up to this project, we have recently conducted a carefully controlled feeding study in 27 postmenopausal women. Sample analysis is currently in progress. The preliminary findings are summarized in the parent CRIS and will be reported at the 2004 Annual meeting of The American Society for Bone and Mineral Research in Seattle, Washington in October, 2004.

Scientific Publications: **Log 115:**

1. Roughead, Z.K., Johnson, L.K., Wagner, J.L. 2004. Effects of soy versus meat protein on calcium retention and bone biomechanical properties in ovarioectomized rats. Federation of American Societies for Experimental Biology Journal. 18:A852-A853. 0000156256

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51000-034-05N **Accession:** 0407993 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 03/01/2004 **Term Date:** 02/28/2009

National Programs: 107 100% Human Nutrition

Title: HISTOMORPHOMETRIC AND BIOCHEMICAL ASSESSMENT OF THE POSSIBLE AUGMENTATION OF BONE HEALING AND REMODELING BY BORON

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-4-0038F

Organization Name: UNIVERSITY OF SALTA

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
This report serves to document research conducted under a non-funded cooperative agreement 58-5450-4-0038F between ARS and the National University of Salta, Argentina. Additional details of the research can be found in the report for the parent CRIS 5450-51000-034-00D.

The purpose of this research is to determine whether boron is bioactive in bone formation and thus promotes bone growth and remodeling, which would inform dietary recommendations for supporting bone health. The animal portion of the experiment to determine the effect of boron deprivation on peri-implant bone healing of the tibia and mandibular bone remodeling upon tooth extraction in mice has been completed. Tissues obtained from the animals are currently being processed for histological examination.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51530-009-00D **Accession:** 0408299 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 04/03/2004 **Term Date:** 04/02/2009

National Programs: 107 100% Human Nutrition

Title: MICRONUTRIENT ROLES IN PHYSIOLOGY AND HEALTH

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**
Suboptimal dietary intakes of essential micronutrients have been statistically associated with chronic disorders such as obesity, diabetes, cardiovascular disease, depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals (e.g., calcium, copper, iron, magnesium, zinc) are less than recommended in many segments of the U.S. population and that mild-to-marginal deficiencies in these and other micronutrients are particularly likely in at-risk and underserved groups (e.g., women, children, elderly, minorities). Unfortunately, controlled studies of the relationships between micronutrients and chronic disease are few and the roles of potential mediating factors such as age, sex, body composition, special diets, lifestyle and genetic factors are poorly understood. For most micronutrients, the potential health benefits and mechanisms of action for physiological (healthy body weight and composition, energy metabolism, brain and cardiac function) and psychological (cognition, emotional and social adjustment, school/work performance) function have not been determined.

This project seeks to improve health and enhance quality of life by determining for healthy and at-risk populations micronutrient intakes that optimize physiological and psychological development, function and health. Specific project objectives are to: (1) develop new functional bases for establishing mineral element requirements; (2) identify mechanisms of action; and (3) determine the influence of mediating factors on mineral element requirements. This project is directly related to the following major components of the National Program Action Plan for Human Nutrition (107): Nutrient Requirements; Relationship between Diet, Genetics and Lifestyle and the Prevention of Chronic Disease; and Health-Promoting Interventions Strategies for Targeted Populations. The research addresses priority objectives including (1) determining functional markers of mineral intakes and status; (2) identifying mechanisms of action of mineral elements; (3) determining the influence of genetic, environmental and lifestyle factors on obesity and prevention of chronic disease; (5) identifying dietary intervention strategies effective with minority populations; (6) characterizing the role of mineral elements in achieving and maintaining optimal physiological and psychological development, function and health.

Controlled studies generate new knowledge to use in making recommendations for dietary intakes that promote optimal development, function and health throughout the life span. Dietary intakes and biochemical indices of mineral status are related to physiologic (e.g., healthy body weight and composition, physical fitness, energy metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine importance of specific minerals for optimal function and development. A mobile field laboratory, community studies facilities, and a residential metabolic unit are used

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to conduct survey, supplementation, fortification, and controlled feeding studies with healthy and at-risk groups (e.g., school-aged children, rural elderly, minorities). Randomized controlled trials evaluate the effects of feeding graded dose amounts of minerals, such as iron, zinc, copper and magnesium, in the context of mediating factors (e.g., genotype, controlled stressors). Animal and cell culture studies enhance the efficacy of human studies and help determine the mechanisms of action of functional outcomes.
This project provides experimentally-derived information needed to establish recommendations for dietary intakes of mineral elements throughout the life span and to help American consumers choose foods that optimize physical and mental performance, social and emotional adjustment, and prevent or ameliorate chronic diseases such as obesity, diabetes, depression and dementia. Resulting information is also useful for evaluating food assistance programs, special diets, the efficacy of taking dietary supplements, and the potential benefits of value-added foods to maintain health and well-being. Primary customers for the products of this research are agricultural and commodity groups, the food industry, supplement manufacturers, policy makers, health and nutrition professionals and the general public.

2. **List the milestones (indicators of progress) from your Project Plan.**

Year 1 (FY 2004-2005)
- Plan, obtain institutional and school board approvals, and initiate for participation of children and adolescents to determine relationships among zinc and iron nutrition and cognitive function, psycho-educational performance, body composition and growth.
- Plan project and obtain approvals to initiate recruitment of healthy elderly living in institutionalized environments to participate in a cross-sectional epidemiologic study of nutrition, health and function.
- Plan and initiate study of the effects of dietary zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity.
- Plan project and obtain approvals to initiate an observational study to determine the nutrient composition of foods in the food assistance programs and traditional foods on American Indian reservations.

Year 2 (FY 2006)
- Conduct study to determine in children and adolescents relationships between zinc and iron nutrition and cognitive function, psychoeducational performance, body composition, growth, and physical fitness at first site.
- Plan project and initiate study of effects of dietary magnesium depletion on neurological and psychological functions of postmenopausal women.
- Conduct observational study of relationships among zinc, copper and magnesium nutrition and physical and mental health of healthy elderly.
- Plan project and initiate study of effects of different body composition phenotypes of rats on copper and zinc metabolism with increased physical activity in rats.
- Analyze samples from study of effects of zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity; report results.
- Complete the nutrient composition survey of components of food assistance program and include traditional Indian foods.
- Plan and initiate study of interaction of dietary boron and essential fatty acids in rats.
- Plan weight loss study of obese humans and recruit subjects.

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Year 3 (FY 2007)
- Conduct study of zinc and iron supplementation in children and adolescents at second site.
- Conduct study of magnesium intake and neurological and psychological functions of postmenopausal women.
- Plan and initiate study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.
- Develop nutrient database of commodity and traditional Native foods.
- Initiate community focus groups to develop appropriate menus including traditional foods to prevent obesity and diabetes in American Indians.
- Analyze samples and data from observational study of the elderly; report results.
- Conduct weight loss and weight maintenance study.
- Plan experimental protocol for study of effects of graded zinc intake on adaptation to increased physical activity in humans.

Year 4 (FY 2008)
- Conduct study of zinc and iron supplementation in children and adolescents at third site.
- Analyze samples and data, and report results of magnesium study in postmenopausal women.
- Analyze samples, analyze data and report results of low zinc effects on physical training.
- Conduct nutritional intervention study of the effects on mental and physical functions of the elderly.
- Conduct study of effects of traditional Native foods on prevention of obesity in American Indian children and communities.
- Plan and initiate study of effects of low copper intake on adaptation to increased physical activity in humans.

Year 5 (FY 2009)
- Analyze results and report findings of zinc and iron intervention studies.
- Analyze samples and data, report findings of the mineral intervention study of the elderly.
- Analyze results of obesity prevention trial in American Indian children and communities.
- Analyze samples and data, report findings of the effects of low copper intake on adaptation to increased physical activity.

3. **Milestones:**

Year 1 (FY 2005)
Mineral Nutrition of Children and Adolescents

- Develop experimental protocol, initiate contacts with participating institutions, obtain institutional and school board approvals, and initiate study to determine relationships among zinc and iron nutrition and cognitive function, psycho-educational performance, physical performance, body composition and growth in children and adolescents in target location (greater Grand Forks, North Dakota and Minnesota American Indian Reservations or Brownsville, TX).

Survey of Nutrition and Function in Healthy Institutionalized Elderly
- Develop experimental protocol, initiate contacts with participating institutions, obtain institutional and administrative approvals, and initiate a cross-sectional survey of nutrition, health and function in healthy elderly living in institutionalized and non-institutionalized environments.

Zinc and Copper Nutrition: Effects on Adaptation to Physical Training in Rats with

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Different Phenotypes for Running
- Develop experimental protocol, conduct pilot study to verify that the breeds of rats respond as anticipated to copper and zinc restriction. Conduct training study.
- Establish analytical methods for DNA microarray to measure expression of oxidative phosphorylation genes.
Nutrient Composition of Foods Available in Food Assistance Programs and Traditional Foods on Reservations
- Establish collaboration with Nutrient Composition Lab at BHNRC for chemical analysis of commodity and traditional American Indian foods.
- Develop plan, establish cooperative relationships with reservations, gain tribal approvals for participation, develop computerized data base for management of nutrient data, start the collection of foods.
Effects of Dietary Boron Restriction on Brain Lipid and Neurotransmitter Contents
- Complete a study in progress to determine if boron restriction in rats influences fatty acid composition of brain phospholipids, prostaglandin and catecholamine contents, and affect eye ultrastructure and brain function.
Effects of Magnesium Restriction on Inflammatory Responses.
- Plan and initiate a study to determine whether magnesium deficiency induces inflammatory responses that lead to a neuronal dysfunction by altering neuropeptide release and prostaglandin formation in healthy postmenopausal women.

Year 2 (FY 2006)
Mineral Nutrition of Children and Adolescents - Intervention Study

- Conduct survey study in selected location (greater Grand Forks, North Dakota and Minnesota American Indian Reservations or Brownsville, TX).
- Analyze biological samples, determine relationships among nutritional indicators and mental and physical functional measures.
Survey of Nutrition and Function in Healthy Institutionalized Elderly
- Conduct study in approved institutions; analyze samples and determine relationships among nutritional and functional measures.
Effects of Magnesium Restriction on Inflammatory Responses
- Conduct study.
Zinc and Copper Nutrition: Effects on Adaptation to Physical Training in Rats with Different Phenotypes for Running
- Complete physical training study; perform chemical analyses; analyze data; report results.

Nutrient Composition of Foods Available in Food Assistance Programs and Traditional Foods on Reservations
- Transfer data from BHNRC to GFHNRC, develop computer data base and deliver it to participating Indian reservation dietary and health promotion leaders.
Effects of Differences in Body Composition Phenotypes on Copper and Zinc Metabolism with Exercise Training
- Develop experimental protocol and initiate exercise training study.

Year 3 (FY 2007)
Mineral Nutrition of Children and Adolescents - Intervention Study

- Based on findings of the observational study, develop experimental protocol, prepare and receive approval from institutional approval boards in target location (greater Grand Forks, North Dakota and Minnesota American Indian Reservations or Brownsville, TX) for the longitudinal study.
Nutrition and Function in Healthy Institutionalized Elderly - Intervention Study
- Based on findings of the observational study, develop experimental protocol,

prepare and receive approval from host institution(s) and institutional review boards.
- Conduct study.
Effects of Magnesium Restriction on Inflammatory Responses
- Analyze samples, then data; report results.
Obesity Prevention in American Indian Children
- Initiate focus group discussions on reservations, develop experimental protocol, prepare and receive approval from institutional and tribal review boards.
- Use nutrient data-base of commodity and traditional foods in discussions with tribal representatives to develop appropriate dietary approaches to prevent obesity in children using the school breakfast and lunch programs as well as community actions.
- Conduct focus group discussion to develop action plan for increasing physical activity in the community with an emphasis on children.
Effects of Differences in Body Composition Phenotypes on Copper and Zinc Metabolism with Exercise Training
- Complete physical training study; perform chemical analyses; analyze data; report results.
Dietary Copper Affects Adaptation to Increased Physical Activity of Out-Bred Rats with Different Phenotypes for Aerobic Capacity
- Develop experimental protocol and initiate exercise training study.

4. **What were the most significant accomplishments this past year?**

Survey of Northern Plains Indian Physical and Mental Health

We compiled a report, entitled Northern Plains Powwow Health and Nutrition Survey: Report to Participating Communities, that summarized findings for each of five American Indian tribes participating in a two-year study to evaluate culturally appropriate research methods and to characterize basic relationships among mental and physical health, nutrition, physical activity, and social and cultural factors in Northern Plains Indians living on North Dakota's four reservations and one Minnesota reservation. Study data were collected using a mobile research laboratory that traveled to 8 pow-wows and health fairs held on the reservations. Preliminary analyses indicate that depression in adult Northern Plains Indians is strongly related to food insecurity and moderately related to less exercise, higher body mass index, and poorer physical health and diet, all moderated by gender. Impact: Findings per se and the sharing of them with tribal leaders and health care providers serve as a foundation for future studies to determine specific nutrient needs and the impact of diet and activity on health issues endemic to American Indian communities, with the goal of implementing effective, culturally appropriate, community-based interventions to improve health and quality of life. This study has also help develop key relationships between regional American Indian communities and the USDA, critical to the success of future nutrition studies in this under-served population group.

B. Other Significant Accomplishments.

Dietary Copper Restriction Decreases the Activity of a Key Enzyme in Muscle Energy Metabolism

Cytochrome c oxidase activity in skeletal muscles composed of different fiber types was reduced in rats fed diets containing copper in concentrations ranging from severe, moderate, mild and adequate. This enzyme plays a key role in aerobic energy production. Impact: This finding suggests that skeletal muscle may be a previously

unrecognized site that requires copper, not only for protection against oxidative damage but also for regulation of energy metabolism.

C. Significant activities that support target populations.

The study reported above in section 4.A. is an example of a successful partnership between American Indians, tribal nations, and the USDA, ARS. The reported research has fundamental interest of the American Indians because of the extraordinary high rates of obesity, diabetes and other chronic diseases in this under-served group. Planned activities within this research project will continue to address, develop and evaluate preventive measures to reduce these health problems among American Indians in the Northern Plains.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
The major accomplishment was the development of trust and respect among the American Indian tribes in North Dakota that resulted in the completion of survey of Native American health, nutrition and physical activity and preparation of a report summarizing the findings. The report is being distributed to participating Indian tribes and communities.

A secondary accomplishment was the identification of the specificity of muscle cytochrome c oxidase activity as a marker on copper nutritional status and its potential for future research to determine the interaction of dietary copper restriction and supplementation on adaptation of muscle metabolism to increased physical activity.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
There are no CRADAs, licenses or patents to report.

Transfer of technical information to other scientists occurred through presentations at national and international meetings and professional publications. Knowledge about the health benefits of mineral nutrients was transferred by routine contacts to representatives of industry and policy-making and regulatory federal agencies. Transfer of knowledge to the public occurred through contacts with media representatives and by direct contacts with the public.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
Popular Press
Bliss, R. M. May 2004. Lack Energy? Maybe Its Your Magnesium Level. Agricultural Research Magazine. 52(5).p.8-9.

Bliss, R. M. July 2004. Breaking Barriers to American Indian Nutrition Research. Agricultural Research Magazine. 52(7).p.18-19.

In Diabetes Today. July 2004. Breaking Barriers to American Indian Nutrition Research.

Media Coverage
H. Lukaski summarized research findings that were described in stories on zinc and

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performance in Bicycling, zinc and iron effects on physical performance in Men's Health, magnesium and energy use during low-level exercise in Today's Health & Wellness and Soy News.

F. Nielsen contributed information from his research on micronutrients in magazine stories in New Women and Women's Health magazines.

J. Penland was cited in stories appearing in Shape magazine, Health magazine and Women's World magazine on effects of dietary boron on brain function, and on copper and sleep; in Science Life magazine on minerals and behavior and in the Minot Daily News on mental and physical health and nutrition.

Presentations

Lukaski, H.C. Non-Invasive Assessment of Body Fluids - Impedance Methods. National Association of General Clinical Research Center Bionutritionists (NAGB), Annual Meeting of the National Institutes of Health General Clinical Research Centers, Chicago, IL, April 16, 2004.

Lukaski, H.C. Fluid Status in Health and Disease - Bioelectrical Impedance Vector Analysis. National Association of General Clinical Research Center Bionutritionists (NAGB), Annual Meeting of the National Institutes of Health General Clinical Research Centers, Chicago, IL, April 16, 2004.

Lukaski, H.C. Low Carbohydrate Diets: Fantasy and Facts. Grand Forks Kiwanis Club, Grand Forks, ND, May 24, 2004.

Lukaski, H.C. Zinc and Other Minerals: Roles in Human Physical and Mental Performance. Committee on Military Nutrition, Food and Nutrition Board, Institute of Medicine, National Academy of Science, Conference on Optimization of Nutrient Composition for Assault Rations, US Army Research Institute of Environmental Medicine, Natick, MA, August 10, 2004.

Scientific Publications: **Log 115:**

1. Lukaski, H.C. 2004. Vitamin and mineral status: Effects on physical performance. Nutrition, 20:632-644. 0000161138
2. Lukaski, H.C. 2004. Lipoic Acid. In: Wolinsky, I., Driskell, J.A., editors. Nutritional Ergogenic Aids. New York, NY: CRC Press. p. 411-429. 0000150535
3. Lukaski, H.C., Hall, C.B., Siders, W.A. 2004. Validity of bioelectrical impedance vector analysis (BIVA) to assess fluid change during weight loss [abstract]. Federation of American Societies for Experimental Biology, 18(4):A1112, 729.1. 0000156241
4. Nielsen, F.H. 2004. Boron. In: Merian, E., Anke, M., Ihnat, M., Stoeppler, M., editors. Elements and Their Compounds in the Environment: Occurrence, Analysis and Biological Relevance, Volume 3, Nonmetals, Particular Aspects. 2nd Edition. Weinheim, Germany: Wiley-VCH. p. 1251-1260. 0000134225
5. Nielsen, F.H., Penland, J.G., Newman Jr, S.M. 2004. Dietary boron modifies the effect of changing dietary fatty acid composition on rat behavior and eye mitochondrial morphology. The Federation of American Societies for Experimental Biology Journal. 18(4):A491. 0000156205
6. Nielsen, F.H. 2004. Micronutrients and animal nutrition. Meeting Abstract. In: International Symposium on Micronutrients, New Delhi, India. Paris, France: International Fertilizer Industry Association. CD-ROM. 0000161121
7. Penland, J.G., Gray, J.S., Lambert, P.L., Wilson, E.L., Gonzalez, J., Lukaski, H.C. 2004. Depression in northern plains indians is associated with physical 0000156511

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health and fitness, dietary intakes, food insecurity and cultural identification [abstract]. The Federation of American Societies of Experimental Biology Journal. 18:A516.

8. Roberge, M.T., Finley, J.W., Lukaski, H.C., Borgerding, A.J. 2004. Evaluation of the pulsed discharge helium ionization detector for the analysis of hydrogen and methane in breath. Journal of Chromatography A. 1027:19-23. **0000151817**

9. Yokoi, K., Egger, N.G., Ramanujam, V.M., Alcock, N.W., Dayal, H.H., Penland, J.G., Sandstead, H.H. 2004. Use of oral contraceptives alters zinc kinetic parameters in women [abstract]. The Federation of American Societies of Experimental Biology Journal. 18:A93. **0000164241**

10. Yokoi, K., Uthus, E.O., Penland, J.G., Newman Jr, S.M., Nielsen, F.H. 2004. Nickel deficiency alters eye mitochondrial morphology and impairs brightness discrimination of rats. The Federation of American Societies for Experimental Biology Journal. 18(4):A527. **0000156312**

Approved: ROOS ERIC E **Date:** 09/14/2004

Project Number: 5450-51530-009-01T **Accession:** 0406520 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 10/01/2002 **Term Date:** 09/30/2007

National Programs: 107 100% Human Nutrition

Title: DETERMINATION OF THE EFFECTS OF BUCKWHEAT ON MANAGEMENT OF PRE-DIABETES AND NON-INSULIN DEPENDENT DIABETES (NIDDM) IN ANIMAL MODELS

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0402

Organization Name: MINN-DAK GROWERS, LTD.

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
   A. None.
   B. None.
   C. None.
   D. This report serves to document research conducted under trust agreement # 58-5450-3-0402 between ARS and Minn-Dak Growers, Ltd. Additional details can be found in the report for CRIS 5450-51530-008-01T.

   Male, Zucker Diabetic Fatty (ZDF) rats were matched by body weight, randomized by group and fed a purified diet (AIN-93) supplemented with a standard dose of fagopyritols (15 mg/kg body weight) from cooked farinetta or fagopyritols isolated from raw farinetta during a 14-week period. After four weeks of adaptation to the diets, blood was sampled weekly for determinations of cholesterol, triglycerides, glucose, insulin, and glycated hemoglobin. Because the ZDF rat progressively develops type 2 diabetes, blood lipid and glucose concentrations increased and insulin decreased significantly in all groups throughout the study. Compared to the basal, non-supplemented purified diet, rats fed fagopyritols and cooked farinetta had significantly reduced glycated hemoglobin at 12 weeks. Fagopyritol treatment had no other effect on the measured variables. The lack of an effect on circulating glucose and insulin concentrations is explained by inability to restrict food intake prior to blood sampling in this animal model because of its advanced diabetes. These findings indicate a beneficial effect of fagopyritols, either from cooked buckwheat or as extracts added to food, on long-term glucose control in a model of type 2 diabetes. They provide limited support for continued research to identify health-promoting effects of buckwheat in obesity and diabetes.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
   See 4D above

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
   FY 2005: Studies will be initiated to determine the effects of fagopyritols on the prevention of type 2 diabetes. One experiment will utilize Zucker Fatty (Zucker fa/fa) rats that will be fed graded doses of fagopyritols from food (buckwheat) and

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extracts added to the diet on oral glucose tolerance and insulin sensitivity. Potential mechanisms of action will investigated including glucose transporters in peripheral tissues, insulin signaling systems and insulin secretion rates.

FY 2006: If the proposed rodent studies reveal that buckwheat has beneficial effects on glucose and insulin metabolism, planning for a study of buckwheat in amelioration of pre-diabetes will be initiated.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
None

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/28/2004

MICRONUTRIENT ABSORPTION AND METABOLISM

MANAGEMENT UNIT

5450-020-00

Project Number: 5450-51000-031-00D **Accession:** 0404974 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 02/22/2002 **Term Date:** 07/20/2004

National Programs: 107 100% Human Nutrition

Title: ROLE OF DIETARY MINERALS ON GENE EXPRESSION, CELL CYCLE AND MOLECULAR MECHANISMS IN CANCER RISK

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

Improving the diet by increasing the consumption of whole grains, fruits and vegetables may decrease the incidence of cancer by 30-40%. Although fiber, vitamins and phytochemicals have received the most attention as chemopreventive components of a diet rich in grains, fruit and vegetables, minerals also may be important chemopreventive components. For example, human epidemiologic and supplementation studies, as well as extensive animal studies, have shown the efficacy of selenium in cancer prevention. Food contains different chemical forms of selenium as well as other dietary constituents which will influence the chemopreventive effect of selenium. Furthermore, recent studies suggest that dietary copper protects against colon cancer in several animal models. Other dietary minerals may be beneficial but their role in cancer prevention has not been thoroughly investigated. Mammary, colon and prostate cancers are the main types of cancer which are influenced by dietary factors. A key to understanding the relationship between optimal mineral intake and cancer is determining the effects of mineral intake on cellular processes such as gene expression, oxidative stress, apoptosis and signal transduction. Studies are and will be conducted to determine whether mineral elements such as selenium, copper, and zinc affect biomarkers of carcinogenesis, including carcinogen-induced aberrant crypt formation (a preneoplastic lesion for colon cancer), carcinogen-DNA adduct formation, oxidative status, selenoprotein and detoxifying enzyme activities, DNA methylation and tumor development. Min (multiple intestinal neoplasia) mice will be used to study the effects of trace minerals on the pathogenesis of intestinal cancer in a genetic model for cancer susceptibility. These mice contain a mutation in the murine homolog of the human APC gene and develop spontaneous tumors throughout the intestine. Several observations implicate a role for altered DNA methylation in cancer pathogenesis: the global level of DNA methylation is generally lower but there is gene specific hypermethylation and DNA methyltransferase activity is usually higher in tumor cells than in normal cells. Global DNA methylation, gene-specific DNA methylation, methyl metabolism and DNA methyltransferase activity will be evaluated in colon-derived human cells cultured in medium containing different chemical forms of selenium and different concentrations of folate, iron, or zinc and in animals fed diets containing different amounts of selenium, folate, iron or zinc. To determine the mechanisms for the chemopreventive effects of selenium and copper against colon cancer, gene specific macroarrays will be utilized and the effects of copper and selenium on signal transduction pathways for apoptosis and regulation of the cell cycle will be examined in cultured cells. The biological activity of zinc transcription factors will be studied using electrophoretic mobility shift assays or reporter gene constructs. Controlled human feeding studies and/or supplementation studies have and will be conducted to determine whether trace

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minerals shown to affect carcinogenesis in animal and cell culture models affect cancer susceptibility in humans. Humans will be fed different diets and fecal water will be analyzed for cytotoxicity, apoptosis, genotoxicity, free radical production and alkaline phosphatase activity. Lymphocytes will be analyzed for DNA methylation, expression of cancer related proteins and measures of oxidative stress/status. Serum from animals or humans fed different concentrations of trace minerals will be used in cell culture systems to investigate cancer susceptibility.

Cancer is the second leading cause of death in the United States. It has been estimated that the cost for the treatment and care of this disease is approaching $200 billion per year. In addition to the economic impact, the development of cancer may prevent many from enjoying life to its fullest. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 30-40% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake are factors that can affect cancer susceptibility. Thus, providing information about requirements and factors that affect those requirements of mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

This research is related to National Program 107, Human Nutrition. The research addresses Performance Goal 3.1.1 of the National Program Action Plan: Human Nutrition Requirements. This research is relevant to Component 1: Nutrient Requirements because one of the priority objectives is to adapt current methods or develop new methods to identify specific disease preventing bioactive dietary factors and elucidate their mechanisms of action. Another priority objective is to use the biomarkers as screening tools to identify the specific bioactive factor(s) responsible for the effects. This research is also relevant to Component 2: Diet, Genetics, Lifestyle and the Prevention of Obesity and Disease. This research will identify the nutrient-relevant influences on gene expression that have consequences on human health and disease.

Several trace minerals have been demonstrated to reduce the risk of developing several types of cancer, but the mechanism by which this occurs is unknown. These studies will address this problem, and thus are of interest to health professionals and policy makers. Understanding the mechanism by which trace elements inhibit cancer has the potential to impact recommendations of how much of the dietary trace mineral should be consumed daily; this in turn has the potential to impact how the medical establishment approaches cancer prevention and how the food industry prepares and/or fortifies specific foods.

2. **List the milestones (indicators of progress) from your Project Plan.**

Determine the use of fecal water as a biomarker for processes related to carcinogenesis. Genomic and proteomic approaches will be utilized to further define the effect of human fecal water and other trace elements on cell cycle and apoptosis in cultured cells, and to determine the impact of mineral status on the biological activity of zinc finger transcriptional factors, which could lead to the identification of molecular bio-marker gene candidates.

Determine in humans the effects of consumption of broccoli high in sulforaphane and selenium on oxidative status and biomarkers of cancer susceptibility.

Determine in animal models the effects of different food versus chemical forms of selenium on carcinogen-DNA adduct formation in the colon and prostate.

Conduct animal studies to determine the effect of dietary selenium and zinc on the zinc-containing selenoprotein SelR.

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Search for genes with altered methylation patterns as a consequence of selenium status (chemical form selenium and dietary concentration of selenium).

Study the anti-carcinogenic benefit of buckwheat in the aberrant crypt model of colon cancer.

Conduct studies using siRNA knockdown of various selenoproteins. These studies will include the use of single and multiple knockdowns in cell culture and will be used to determine the importance of these various selenoproteins in carcinogenesis.

Cell culture studies will be initiated to determine how quinone oxidoreductase and thioredoxin reductase, two enzymes regulated in part through the antioxidant response element (ARE), are coordinated in response to oxidative stress.

Conduct animal studies to determine the effect of cross-breeding mice with a knocked out selenoprotein (most likely GPx) to mice that are prone to a specific cancer (mammary cancer, for example). These studies will definitively ascertain the importance of the selenoprotein for a particular cancer.

3. **Milestones:**

A. List the milestones that were scheduled to be addressed in FY 2004. How many milestones did you fully or substantially meet in FY 2004 and indicate which ones were not fully or substantially met, briefly explain why not, and your plans to do so.

As stated, this project is scheduled to be replaced by another project plan (5450-51000-036-00D) which continues work in a similar area but has not been certified. The following FY2004 milestones are from 5450-51000-031-00D, the project being terminated.

In FY 2004, sample collection of the two human feeding studies will be completed: 1) A controlled human feeding study investigating the effect of wheat cereals containing various concentrations of selenium on selenium status, antioxidant status, cytotoxocity of fecal water and measures of colonic fermentation. 2) A controlled feeding study investigating the effects of consumption of broccoli high in sulforaphane and selenium on oxidative status and biomarkers of cancer susceptibility. These studies were conducted in FY2004.

Animal studies will be conducted investigating the effects of different food versus chemical forms of selenium on carcinogen-DNA adduct formation in the colon and prostate. These studies were conducted but prostate was not used as a target tissue. In the process of evaluating our research we decided to focus on one cancer type - colon cancer.

Animal studies will be conducted to determine the effect of dietary selenium and zinc on SelR and Msra. These findings will be correlated with markers of oxidative stress and biomarkers of cancer susceptibility. These studies were completed.

A search for genes with altered methylation patterns as a consequence of selenium status (or form of dietary selenium) will be started. This screening has begun by using restriction landmark genomic scanning.

Genomic and proteomic approaches will be utilized to further define the effect of human fecal water and other trace elements on cell cycle and apoptosis in cultured cells, and to determine the impact of mineral status on the biological activity of

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zinc finger transcriptional factors, which could lead to the identification of molecular bio-marker gene candidates. All of this has been accomplished except work on the zinc finger transcription factors. This aspect of the studies was eliminated during the development of our new research plan.

Buckwheat is a cereal crop that contains rutin and quercitin, both powerful antioxidants, and preliminary studies have shown that buckwheat reduces the incidence of some cancers. We will study the anti-carcinogenic benefit of buckwheat in the aberrant crypt model of colon cancer. A bioavailability study conducted in FY2004 indicated selenium in buckwheat to be poorly available. We need to determine the reason for this. Therefore, a cancer study is premature and has not been conducted.

B. List the milestones that you expect to address over the next 3 years (FY 2005, 2006, & 2007). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

The following milestones are from the project plan that will replace this project which is being terminated. The replacement project (5450-51000-036-00D) has not been certified but continues work in the same area.

Year 1 (FY 2005)

Initiate and optimize human colon cell culture studies to determine the antioxidant ability related to cellular selenium and whether cellular selenium status regulates the cyclin-dependent kinase pathway through the c-Myc gene. The conditions for these experiments have been defined and initial studies are ongoing.

Initiate and optimize experiments to determine whether methylselenol causes cell cycle arrest, affects cancer cell invasive ability, and induces differential gene expression in the cyclin-dependent kinase pathway. The conditions for these experiments have been defined and initial studies are ongoing.

Develop constructs and conduct experiments to determine whether thioredoxin reductase (TR) is regulated by selenium availability (dose and chemical form) as well as by ARE inducers and by oxidative stress. The cell culture model used to study TR regulation has been developed and validated; reporter gene constructs have been made and tested.

Determine whether phytochemicals in broccoli act synergistically with selenium in increasing TR activity. We have conducted preliminary studies with chemicals in broccoli, and these initial results show that sulforaphane, a glucosinolate breakdown product, accounts for most of the transcriptional activation of TR by broccoli extracts. Experiments to confirm and extend these findings are ongoing.

Complete the animal portion of the experiment to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression. Initiate metabolite and enzyme assays. The animal portion of one study that ascertained the effect of concentration of selenium has been completed. Many of the metabolite and enzyme assays are completed. Another experiment that used several chemical forms of selenium was undertaken. The metabolite and enzyme analyses for this experiment are ongoing.

Complete animal portion of experiment to study the interaction between selenium and folate. Initiate metabolite and enzyme assays. Begin restriction length genome

scanning (RLGS) on select samples. The animal work has been done and many of the enzyme and metabolite assays are complete. The RLGS work is scheduled to start this fall.

Complete and report RLGS on initial Ames dwarf mouse study. The preliminary results from RLGS indicated that several genes were differentially methylated in Ames versus wild type controls. These genes were further studied by bisulfite sequencing but because of possible confounding effects as a result of polymorphisms the results are inconsistent. Thus, additional samples will be run.

Year 2 (2006)

Finish data collection on the colon cell culture studies designed to determine whether cellular selenium status regulates the cyclin-dependent kinase pathway through the c-Myc gene.

Finish data collection on the experiments designed to determine whether methylselenol causes colon cell cycle arrest and induces differential gene expression in the cyclin-dependent kinase pathway.

Perform bioinformatics search/study to understand gene data derived in the above cell culture experiments.

Prepare extracts from plants (other than broccoli) and conduct the studies to determine other compounds that transcriptionally upregulate the TR ARE independent of selenium.

Complete analyses from the experiment designed to determine the effect of form and concentration of selenium on methionine sulfoxide activity and expression.

Complete enzyme and metabolite analysis (including RLGS) from experiment designed to study the interaction between selenium and folate.

Initiate RLGS on Ames dwarf mice and age-matched wild type controls.

Year 3 (2007)

Optimize the colon cell culture conditions and start a study to determine how cellular selenium status regulates the mitogen-activated protein kinase pathway.

Optimize the colon cell culture conditions and start a study to test the hypothesis that selenium-induced apoptotic signaling is different in normal versus transformed cells.

Determine whether knockdown of TR will have minimal functional consequence for the cell because related ARE-regulated antioxidant systems will be compensatorily upregulated.

Initiate preliminary experiments to test the hypothesis that simultaneous knockdown of TR and a second ARE-regulated protein (ferritin) will severely damage the ARE-regulated antioxidant network and result in severe cellular damage.

Complete methylation assays and gene identification from RLGS in the selenium-folate and Ames dwarf mice studies.

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Initiate RLGS study in Ames mice fed various forms of selenium.

4. What were the most significant accomplishments this past year?

A: Single most significant accomplishment during FY 2004 year (one per Research Project):

Selenium-enhanced broccoli extracts reduced oxidative DNA damage in cultured cells.

In cell culture studies, extracts of broccoli were found to significantly reduce the DNA strand breaks. If not repaired, these breaks can result in non-viable DNA; accumulation of which can result in cancer. When the experiment was repeated with extracts of broccoli that was enriched with selenium, almost twice as much protection against DNA strand breaks was found when compared to extracts with normal broccoli. These results demonstrate the ability and importance of selenium to function as an antioxidant and to provide a possible mechanism for its anticarcinogenic properties.

B. Other significant accomplishment(s), if any:

Selenium and copper interact to influence cell cycle progression.

One of the proposed mechanisms for the anticarcinogenic effects of selenium is that selenium induces the death of damaged cells (apoptosis). Previous studies using cultured cells have demonstrated that copper can inhibit selenium-induced apoptosis; however, the exact nature of this interaction is not fully understood. We performed cell culture experiments in which selenium and copper, alone or together, were added to the growth media of the cells. Data from these experiments suggest that copper interferes with the uptake of selenium into cells resulting in less apoptosis. These results are beneficial in that they are helping us understand selenium's anticarcinogenic mechanism and how other compounds (i.e., copper) impact this mechanism.

Sulforaphane in broccoli may enhance antioxidant defense through thioredoxin reductase.

The enzyme thioredoxin reductase (TR) plays a significant role in oxidation-reduction reactions and thus, is important in anti-oxidant defense mechanisms. This enzyme is a selenium-dependent protein. We conducted cell culture studies and preliminary results show that sulforaphane, a compound found in broccoli, causes increased synthesis of TR. Greater availability of TR means that the cells have an increased ability to defend against oxidative stress.

Selenium involved with repair of oxidative damage to proteins.

Recently, it was found that the enzyme methionine sulfoxide reductase B (MsrB) is a selenium-containing protein that also contains zinc. This enzyme prevents the oxidation of proteins that contain the amino acid methionine; oxidation of these proteins can result in loss of function. We determined the effects of dietary selenium and zinc on MsrB activity in mice. We found that selenium status significantly affects MsrB, that marginal zinc deficiency has little effect on MsrB. The findings suggest that animals fed marginal or deficient selenium may have impaired capacity to repair oxidized methionine residues in proteins - this could ultimately result in a non-functioning protein. Accumulation of non-functioning proteins can result in increased susceptibility of disease including cancer.

C. Significant activities that support special target populations:

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None.

D. Progress Report opportunity to submit additional programmatic information to your Area Office and NPS (optional for all in-house ("D") projects and the projects listed in Appendix A; mandatory for all other subordinate projects).

None.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

The mechanisms by which the aqueous phase of human feces (fecal water) can positively or negatively affect cell cycle progression in colon cells during tumor development are not completely understood. We studied possible mechanisms with the goal of developing sensitive biological indicators for colon cancer risk by using easily obtainable fecal water. Our results indicate that human fecal water, which can be altered by dietary components, can induce genes that affect cell division and may be important in protection against colon cancer. One of these genes appears to be uniquely and highly sensitive to human fecal water; thus, it is being further studied as a possibly marker of colon cancer.

Selenium is a component of several antioxidant enzymes and regulation of some of these enzymes may be linked to the ability of selenium to protect against cancer. Reporter gene constructs of thioredoxin reductase, a seleno-enzyme, were produced at the GFHNRC and inserted into human liver cells that were then incubated with multiple compounds associated with protection against oxidative stress. Reporter genes were activated in a manner that suggested the presence of a regulatory gene sequence called the Antioxidant Responsive Element (ARE); mutation and gel shift analysis confirmed the presence of this sequence. This study demonstrates the seleno-enzyme thioredoxin reductase is part of a large family of genes that are coordinately regulated in times of oxidative stress by the Antioxidant Responsive Element, and thus thioredoxin reductase may help decrease oxidative damage-induced carcinogenesis.

Many observations suggest an important role for DNA methylation in cancer pathogenesis. We demonstrated that dietary selenium and folate interact to affect DNA methylation and one-carbon metabolism. Our studies showed that selenium and folate interacted to influence one-carbon metabolism and cancer susceptibility such that the number of aberrant crypts and the concentrations of plasma homocysteine and liver S-adenosylhomocysteine were the highest and the concentrations of plasma folate and liver S-adenosylmethionine and the activity of liver methionine synthase were the lowest when rats were fed deficient folate and supplemental selenium. These results suggest that understanding this interaction between selenium and folate may help determine the mechanism of cancer protection afforded by folate and high dietary or supplemental selenium and, that when studying folate, selenium status should be monitored and, vice versa, when studying selenium, folate status should be monitored.

Demonstrated that selenium is effective in the prevention of chemical carcinogen-induced aberrant colonic crypt foci, preneoplastic lesions indicative of colon cancer. Aberrant crypt foci development was inhibited in a dose dependent fashion that was also dependent on the form of selenium. The same pattern of inhibition of aberrant crypts by different chemical forms of selenium was also noted for the inhibition of carcinogen-induced DNA-adducts, suggesting that the inhibition of DNA-adduct formation may be a mechanism for the overall chemopreventive effect of selenium.

Demonstrated that selenium from high-selenium broccoli was more effective than salt or amino acid forms of selenium for prevention of colon cancer. Found that high-selenium broccoli sprouts were as efficacious as broccoli florets for prevention of

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colon cancer.

Familial adenomatous polyposis (FAP) is a disease that has been linked to changes in the APC gene known as mutations; individuals possessing these mutations develop numerous intestinal polyps (precancerous lesions) at an early age. Min mice (multiple intestinal neoplasia mice) carry a mutation in what is equivalent to the human APC gene and develop intestinal tumors similar to those found in patients with familial adenomatous polyposis syndrome and are therefore a good model for the investigation of the effects of dietary alterations on genetic susceptibility for intestinal cancer. Min mice that were fed selenium-enriched broccoli had a significantly lower small intestine tumor incidence and a significantly lower small intestine tumor burden than animals fed adequate dietary selenium as selenite. These findings suggest that inadequate selenium-enriched broccoli can decrease tumor development in a genetic model for human cancer.

Several observations implicate a role for altered DNA methylation in cancer pathogenesis: the global level of DNA methylation is generally lower and DNA methyltransferase activity is usually higher in tumor cells than in normal cells. Studies were conducted to determine whether a DNA methyltransferase inhibitor would alter the effect of dietary selenium on the formation of aberrant crypts, a preneoplastic lesion for colon cancer. Animals fed a selenium-deficient diet had a significantly higher number of aberrant crypts than animals fed adequate dietary selenium; however, when animals were injected with a DNA methyltransferase inhibitor, there was a significant reduction in aberrant crypt formation and dietary selenium did not affect aberrant crypt formation. These results suggest that decreased DNA methyltransferase activity may protect selenium deficient animals against colon cancer susceptibility.

The elucidation of the mechanisms by which selenium regulates the cell cycle can lead to a better understanding of the nature of selenium's essentiality and its role in disease prevention. The effects of selenium deficiency or adequacy on HL-60 (a human lymphocytic cell line) cell progression were examined in serum-free media. Cell cycle analysis revealed that selenium deficiency inhibited cell cycle progression; gene analysis revealed that selenium deficiency decreased the mRNA expression of many cell cycle regulatory genes; and phosphorylation analysis demonstrated that selenium deficiency decreased the phosphorylation state of total cellular protein. Collectively, these results suggest that selenium is critical for human lymphocyte cell division, growth and prevention of cell death.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
   None.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
   None.

Scientific Publications: **Log 115:**

1. Davis, C.D., Uthus, E.O. 2003. Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global dna methylation and one-carbon metabolism in rats. Journal of Nutrition. 133:2907-2914. 0000150074

2. Finley, J.W. 2003. The antioxidant responsive element (ARE) may explain the protective effects of cruciferous vegetables on cancer. Nutrition Reviews. 61 (7):250-254. 0000144908

3. Hintze, K.J., Wald, K., Finley, J.W. 2004. Knockdown of thioredoxin reductase-1 (tr) results in increased measures of cellular oxidative damage [abstract]. 0000156322

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Federation of American Societies for Experimental Biology Journal. 18:A917.

4. Keck, A.S., Finley, J.W. 2004. Selenium from selenium-enriched broccoli is more effective than selenite or broccoli alone for the reduction of h2o2-induced dna strand breaks in hepa 1c1c7 cells. Meeting Abstract. 0000167638
5. Leary, P., Hintze, K.J., Keck, A., Wald, K., Finley, J.W. 2004. Selenium (se) and sulforaphane (sf) interact to reduce oxidative damage in ht-29 cells [abstract]. The Federation of American Societies for Experimental Biology Journal. 18:A916. 0000156329
6. Uthus, E.O., Moskovitz, J. 2004. The specific activity of methionine sulfoxide reductase in mice is significantly affected by dietary selenium but not zinc. Federation of American Societies for Experimental Biology Journal. 18:A916. 0000156584
7. Uthus, E.O. 2003. Arsenic essentiality: a role affecting methionine metabolism. Journal of Trace Elements in Experimental Medicine. 16:345-355. 0000147626
8. Zeng, H. and Botnen, J.H. 2004. Copper may interact with selenite extracellularly in cultured HT-29 cells. Journal of Nutritional Biochemistry. 15:179-184. 0000149800
9. Zeng, H. Genomic and Proteomic Techniques and Their Application in Selenium Research. 2003. Current Pharmacogenomics. v.1. p. 59-65. 0000140536
10. Zeng, H., Botnen, J.H. 2004. Copper inhibition of selenite-induced cell cycle arrest appears to be extracellular. Journal of Federation of American Societies for Experimental Biology. A914. 0000156264

Approved: ROOS ERIC E **Date:** 09/14/2004

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 01/15/2004 **Term Date:** 01/14/2009

National Programs: 107 100% Human Nutrition

Title: MINERAL UTILIZATION AND BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS AND AGRICULTURAL PRACTICES

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

This is the annual report for the new OSQR-approved project, 5450-51000-035-00D, that replaces a related project, 5450-51000-032-00D (see separate annual report terminating that project).

Current trends and proposed changes in the US diet can substantially influence the mineral nutrient status of the population. Such changes include: a) an emphasis on plant-based diets with limited intake of red meat, b) mineral fortification or supplementation of diets proposed by health care professionals, or independently initiated by producers, and c) new technologies to produce genetically- or chemically- modified foods, or to stabilize and enhance the bioavailability of nutrients added to foods. The changes that may affect the mineral contents and/or the absorption and utilization (bioavailability) of dietary minerals, or disrupt the balance among interacting dietary minerals must be identified. Effective approaches such as dietary modifications, fortification/supplementation strategies, and agricultural and food production approaches must be identified to enhance human mineral nutrition and promote health.

The project has 3 objectives: (1) Determine how shifts in agricultural and dietary practices, such as the availability of functional/genetically modified foods and emphasis on plant-based diets with reductions in meat consumption will impact the intake, bioavailability, and dietary requirements of minerals. (2) Determine the effectiveness of current and proposed mineral fortification/supplementation practices for improving mineral nutrition while avoiding excessive or imbalanced mineral intakes. (3) Determine the mechanisms of uptake, transport, and retention of food minerals and how mineral nutritional status influences these mechanisms to impact the bioavailability of essential minerals, non-nutritive metals, and other food components.

This project will evaluate modifications that can enhance trace element nutrition, with emphasis on selenium (Se), iron (Fe), zinc (Zn), and copper (Cu). Agricultural practices that can influence the mineral contents of foods, especially Se, will be evaluated, with assessment of the health properties of such Se-enhanced foods. The influence of reduced dietary Fe and Zn bioavailability will be evaluated in human studies that will help define quantitative mineral needs, elucidate the effect of dietary phytate in combination with dietary Ca fortification, and evaluate the effectiveness of different forms of iron used for food fortification. An algorithm will be developed to assess the Zn bioavailability of diets from generally available food composition data. The basic mechanisms of uptake, transport, and retention of food minerals will be evaluated to determine how mineral nutritional status and intake impact the absorption and metabolism of essential minerals, in interaction with non-nutritive metals and food components.

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This research is directly related to three components of the ARS National Human Nutrition 107 research plan: (1) Human Nutrition Requirements, (6) Health Promoting Properties of Plant and Animal Foods, and (7) Bioavailability of Nutrients and Food Components.

This research will provide critical information about the biological control of mineral absorption, of nutritionally important mineral-mineral interactions, and of bioavailability of minerals from various food and fortification sources. This knowledge is essential for the development of Dietary Reference Intakes, FDA food and dietary supplement regulations, and other U.S. nutrition and food policy guidelines for providing safe and adequate mineral nutrition from the food supply, and will be useful for the agricultural and food production sectors in the development of healthful foods.

The results of these studies will be useful to food producers for the development and promotion of healthy foods, and to health care officials and educators for the development of dietary advice that contributes to optimal nutrition. The ultimate beneficiary is the American consumer through policies and guidelines set by scientists and health care professionals based on these research findings and through transfer of enhanced agricultural products that improve nutrition worldwide.

2. **List the milestones (indicators of progress) from your Project Plan.**
(Because this CRIS project began in January, 2004, and annual reports are prepared in July, the first year's progress will be completely reported in FY2005)

Year 1 (FY2004-5)
Complete 1st yr of wheat & buckwheat Se accumulation studies (1.1)
Complete transgenic wheat accumulation of Se study (1.1)
Develop cells lines and constructs for antioxidants & gene expression study (1.5)
Develop Se speciation methodology (1.1)
Complete interim blood analyses for Fe excretion study & write initial paper (1.9)
Enroll subjects in elemental Fe powder study (2.1)
Complete antibody prep & perform studies on up/down regulation of mineral transport in Caco-2 cells (3.1)
Complete studies on Cu transporter trafficking in Caco-2 cells given high Zn media (3.2)

Year 2 (FY2006)
Complete 2nd yr of wheat & buckwheat Se accumulation studies (1.1)
Complete anti-oxidants & gene expression study (1.5)
Complete 1st yr of organic/conventional foods study (1.1)
Report transgenic wheat study (1.1)
Complete Se speciation in wheat study (1.1)
Develop Zn algorithm & prepare paper (1.7)
Enroll subjects in Zn requirement study (1.6)
Complete elemental Fe powders study (2.1)
Wrap up studies on up/down regulation of mineral transport in Caco-2 cells. Write manuscripts (3.1)
Wrap up studies on Cu transporter trafficking. Write manuscripts (3.2)

Year 3 (FY2007)
Complete feeding portion of human high-Se beef study (1.3)
Report antioxidants & gene expression study (1.5)
Report wheat & buckwheat accumulation studies (1.1)
Complete 2nd yr of organic/conventional foods study (1.1)
Complete high-Se beef and aberrant crypt study (1.3)

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Complete Zn requirement study (1.6)
Report elemental Fe powders study (2.1)
Complete studies on supra-nutritional minerals on Cu, Fe transporters (3.3)
Complete studies on relationship between Cu deprivation & Heph activity (3.4)

Year 4 (FY2008)
Complete analyses for human high-Se beef study (1.4)
Complete differences in bioavailability of mineral nutrients from organic/conventional broccoli (1.2)
Report high-Se beef and aberrant crypt study (1.3)
Complete study of phytate X Ca & Zn bioavailability (1.8)
Report Zn requirement study (1.6)
Complete microencapsulated Fe study (2.2)
Wrap up Cu/Heph/Fe absorption studies. Write manuscripts (3.4)
Complete study on marginal mineral & Cd exposure, analyze data (3.5)

Year 5 (FY2009)
Report human high-Se beef study (1.4)
Complete comparison of high-Se foods aberrant crypt study (1.2)
Report comparison of high-Se foods and aberrant crypt study (1.3)
Report organic & conventional foods study (1.1)
Report study of phytate X Ca & Zn bioavailability (1.8)
Complete final report of Fe excretion data (1.9)
Report microencapsulated Fe study (2.2)
Wrap up studies in objective 3.5. Write manuscripts
Complete new 5-year proposal

3. **Milestones:**

A. Milestones that were scheduled for FY2004-5:

The following milestones scheduled for FY2005 were completed:

Enroll subjects in elemental Fe powder study (2.1). Twenty-eight subjects have completed approximately half of the study protocol.

Complete 1st yr of wheat & buckwheat Se accumulation studies (1.1) This milestone has been successfully completed; we have begun collecting second year samples.

Develop Se speciation methodology (1.1) The selenium speciation method has been developed and initial results have been generated.

Complete antibody prep & perform studies on up/down regulation of mineral transport in Caco-2 cells (3.1). Production of antibodies to the human copper transporter, ATP7b, was completed. Additional antibodies are being obtained privately and commercially.

Complete studies on Cu transporter trafficking in Caco-2 cells given high Zn media (3.2). Studies determined that excess Zn in the media of Caco-2 cells did not affect the expression of the ATP7b protein. New approaches will be taken.

The following scheduled milestones scheduled for FY2005 are in progress:

Complete interim blood analyses for Fe excretion study & write initial paper (1.9). Blood and data analyses are in progress. Sample collection has been completed for sixty-two subjects.

Complete transgenic wheat accumulation of Se study (1.1) Wheat has been successfully transfected, grown to maturity and analyzed. Modifications in the growth procedure have been made and we are repeating some aspects of the study.

Develop cells lines and constructs for antioxidants & gene expression study (1.5) This milestone has been partially met, and work is ongoing.

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The following milestones were partially completed ahead of schedule (years 3-5):
Complete studies on relationship between Cu deprivation & Heph activity (3.4). This milestone had one study completed and the manuscript written. This remains an active field of research for this CRIS, with evolving new approaches.
Complete study on marginal mineral & Cd exposure; analyze data (3.5). Wrap up studies in objective 3.5. Write manuscripts. One study was completed and the manuscript written; however, it is an ongoing project with other experimental approaches being generated.

B. Milestones to be addressed over the next 3 years (FY2005, 2006, 2007):

The listing of milestones in item 2 above will continue to be addressed on schedule, without major revision for this newly approved project.

4. What were the most significant accomplishments this past year?

A. Single most significant accomplishment during FY 2004:

a) Incorporation of a selenium accumulation gene into wheat. Wheat contains selenium, but the total amount varies depending on the concentration of selenium in the soil. Some plants accumulate large amounts of selenium regardless of the soil selenium concentration, such plants are termed selenium accumulators and the gene responsible for this accumulation has been identified (SeCys methyl transferase, or SecMT). In a project that is collaborative with Dr. Ann Blechl, USDA/ARS, Albany, CA, and Dr. Mike Grusak, USDA/ARS, Houston, TX, SecMT was inserted into wheat, stable cultivars were grown to maturity, and seeds were collected. Wheat was then grown hydroponically in the presence of varying amounts and chemical forms of selenium. All cultivars, transfected and wild type, took up large amounts of selenium, but at high concentrations, the transfected wheat took up more. Analyses of growing conditions have suggested methods of greatly increasing the uptake of selenium by the transfected cultivars. Development of selenium accumulating strain of wheat may someday allow production of wheat with extremely high concentrations of selenium which could be used as a source of supplemental Se or as a selenium-fortificant in cereal grain-based products.

B. Other significant accomplishments:

b) Zinc interference with copper transport appears unrelated to the expression of the human copper transporter, ATP7b. Antibodies to the human copper transporter, ATP7b, were produced, and studies were carried out to test whether excess Zn in the media of cultured Caco-2 cells, a human enterocyte mimic, would affect the expression of the ATP7b protein. Although Zn treatment depressed Cu transport across a monolayer of these cells, the relative expression of ATP7b was not changed. Another possibility for the effect of high Zn on Cu transport is that Zn is affecting the function of the transporter without affecting the concentration of the protein itself. There are 6 metal binding motifs at the N-terminal domain of the protein. It is possible that high Zn is displacing Cu at these binding sites. This will be tested in additional experiments. An understanding of the interaction between copper and zinc absorption will be useful in setting recommendations for balanced dietary intakes of the two elements.

c) Confirmation that copper deficiency reduces iron absorption. Copper deficiency causes iron deficiency anemia in most animal species. It was hypothesized that part of the problem was that copper deficiency inhibits iron absorption from the intestinal tract. However, previous research by others, using insensitive

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techniques, obtained mixed results - some found that iron absorption was reduced in male animals; some found that it was enhanced in female animals. In recent studies, we showed that copper deficiency indeed reduced the intestinal absorption of iron in male rats. To test whether similar effects were produced in female rats, a study was carried out by feeding a test diet labeled with radioactive iron to 28-day post-weanling copper deficient female rats and using whole-body-counting techniques to determine copper absorption. It was shown that copper deficiency does indeed reduce iron absorption by about 30% in female rats. These findings suggest that at least part of the cause of iron deficiency anemia in copper deficient animals of both sexes is a lack of efficient absorption of dietary iron. An understanding of the role of copper in iron absorption will be useful in setting recommendations for balanced dietary intakes and addressing the causes of nutritional anemias.

d) Activity of hephaestin, a copper-dependent iron oxidase, was enhanced in copper-deficient rats. Copper deficiency causes iron deficiency in most animal species and part of the effect is thought to be the inhibition of a copper-dependent iron oxidase (Hephaestin) in the intestinal cells that is required to oxidize iron before it can be absorbed. A study was conducted at the Grand Forks Human Nutrition Research Center to determine if the activity of hephaestin is reduced in copper deficiency. The laboratory rat was used as the experimental model. After the rats had been fed a copper deficient diet, and developed signs of iron deficiency, the intestinal epithelial cells were isolated and hephaestin separated on a gel substrate by non-denaturing electrophoresis. An in-gel assay was performed to determine the relative activity of the enzyme. Rather than a decrease in hephaestin activity as expected, activity was enhanced. This occurred in spite of the other signs pointing specifically to a reduction in iron absorption, e.g., increased iron in epithelial cells, low plasma iron, low blood hemoglobin, etc. Further understanding of the role of copper in iron absorption will be useful in resolving the problem of nutritional anemias.

e) Development of methods to determine selenium speciation in foods. Selenium consumption has been demonstrated to reduce the incidence of colon cancer, and the chemical form of the selenium may affect its anticarcinogenic activity. Many plant foods contain selenium but the chemical forms are unknown. Elucidation of the chemical forms of selenium in plant foods will allow us to better predict whether consumption of that food will reduce cancer. The selenium compounds in dried vegetable powder were extracted and analyzed by HPLC coupled to inductively coupled plasma mass spectrometry. Most plants contained salts of selenium, but a few plants contained selenium in methylated forms - forms that may be especially chemoprotective. The method was sensitive enough to be used with plants that contained quite low concentrations of selenium. Development of this methodology will allow us to better predict the bio-activity of selenium from numerous common plant foods.

f) Antioxidant properties of selenium-enriched broccoli. Broccoli consumption is associated with decreased risk of some cancers; selenium consumption is also associated with cancer chemo protection. A potential mechanism for such protection that may be common to broccoli and selenium may be amelioration of oxidative stress. Rat liver cells were used in combination with a test called the COMET assay (a measurement of DNA strand breaks) to measure the resistance of cells to oxidative stress. Extracts of broccoli effectively reduced oxidative stress, and the most active ingredient was sulforaphane, a secondary plant compound. Broccoli that was enriched in selenium was much more effective that normal broccoli for reduction of stress. These results show that broccoli and selenium can protect cells against DNA strand breaks. Because DNA strand breaks are a major initiating event of many

cancers, these results, suggest that some of the cancer protective effects of these substances may be protection against oxidative stress.

g) Elemental iron powders are not equally efficacious as iron fortificants. Elemental iron powders commercially produced in the metallurgy field are commonly used economical sources of iron fortification. These powders minimize adverse chemical reactions in fortified foods, but have not been fully demonstrated to be absorbable sources of iron. In a follow-up to tests of such powders and in cooperation with SUSTAIN (Sharing US Technology to Aid in the Improvement of Nutrition), iron bioavailability of six commercial elemental iron powders was assessed by the rat hemoglobin repletion and slope ratio analysis technique. The tested powders differed considerably in bioavailability, ranging from 10-65%, relative to ferrous sulfate (=100%). This research provides information useful in determining economical and efficacious iron fortification sources to help combat the serious global problem of iron deficiency anemia.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

This new project is a continuation of a project that has investigated copper, iron, selenium and zinc absorption and metabolism. Some highlights include:

- marginal intakes of zinc, iron, and calcium greatly enhanced the accumulation of cadmium in the upper small intestine, leading to a higher accumulation of the toxic metal in the liver and kidneys, which suggests that populations with these nutrient deficiencies are especially susceptible to cadmium toxicity.
- a novel role for selenium in the up-regulation of cell cycle related genes was discovered, that may lead to a better understanding of the essentiality of selenium as a nutrient and its involvement in cancer prevention.
- although Se-methyl selenocysteine was the primary form of selenium in broccoli, methyl selenol was the primary form of Se absorbed by intestinal cells in culture, which may help explain the observed high efficacy of Se from broccoli for preventing colon cancer.
- compared to a conventional farming technique, an organic farming method had limited influence on several nutritional characteristics of broccoli, including trace minerals, multiple individual glucosinolates, primary glucosinolate breakdown products, vitamin C and phenolic acids.
- enhancing the selenium content of broccoli decreased total glucosinolate content, specifically sulforaphane, and changed the phenolic profile, especially reducing the content of hydroxy-cinnamic acids, suggesting that it may not be possible to simultaneously maximize all bioactive ingredients in a food, as enrichment with one compound may cause a concomitant decrease in another.
- elemental iron powders commonly used to fortify staple foods with iron were less bioavailable to rats than iron from ferrous sulfate, and commercial versions differed considerably, suggesting that higher concentrations of these forms may be needed if they are used in international iron fortification programs.
- in a sample of 262 healthy pre-menopausal US women, body iron stores were primarily predicted by a self-estimation of menstrual losses, and less substantially related to the dietary consumption of meat, phytic acid (from whole grains, legumes, and nuts), tea, or iron supplements, suggesting that low iron stores in many US women may be primarily related to increased menstrual loss, rather than poorer diets.
- copper deficiency reduces iron absorption in the rat experimental model by as much as 50%, which in turn, leads to iron deficiency anemia. If similar effects are found in humans, they will strongly suggest that iron deficiency anemia as a world health problem should not be studied without knowledge of the nutritional copper

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2004

status of the population affected. Iron supplementation for iron deficiency signs may be of no benefit without a parallel supplement of copper.

- reduced and electrolytic iron sources were approximately 50 and 85% as effective as ferrous sulfate and 5 mg iron in the heme form was half as effective as 50 mg of iron from ferrous sulfate for improving body iron in premenopausal women.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
None

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
Reeves, P. G. "The Atkins Diet: Worthwhile or Worthless". Grand Forks Herald, February 4, 2004.

Reeves, P.G. Interview with Michiko Shiraishi of the Fiji Press America, Ltd, April, 2004. The article entitled "Will the Low-Carb Craze Change the American Diet?" was featured in 5 local newspapers in Japan, with quotes from Dr. Reeves.

Finley, J. W. "Eat North Dakota Beef; It's Good for You". Grand Forks Herald, May 19, 2004.

Hunt, J.R. "Women's Health Gets Boost at Nutrition Lab. Grand Forks Herald, May 5, 2004.

JR Hunt, "Nature vs. Nurture: Heme and Nonheme Iron Absorption by Carriers of the Genetic Mutation Associated with Hemochromatosis". Presentation in the University of North Dakota Biochemistry and Molecular Biology Seminar Series, Grand Forks, ND, May 6, 2004.

Scientific Publications: **Log 115:**

1. Reeves, P.G., Demars, L.C. 2004. Copper deficiency reduces iron absorption and biological half-life in male rats. Journal of Nutrition. 134:1953-1957. **0000163342**
2. Reeves, P.G., Chaney, R.L. 2004. Marginal nutritional status of zinc, iron, and calcium increases cadmium retention in the duodenum and other organs of rats fed a rice-based diet. Environmental Research. Available: http://dx.doi.org; then enter: 10.1016/j.envres.2004.02.013. **0000153232**
3. Swain, J.H., Newman Jr, S.M., Hunt, J.R. 2003. Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area. Journal of Nutrition. 133:3546-3552. **0000147580**
4. Reeves, P.G. 2004. Varying media zinc, iron, and calcium affect uptake and transport of cadmium caco-2 cells [abstract]. Federation of American Societies for Experimental Biology Journal. 18:A528. **0000156581**
5. Reeves, P.G., Demars, L.C. 2004. Copper deficiency in rats reduces iron absorption and increases the rate of iron loss from the body [abstract]. Federation of American Societies for Experimental Biology Journal. No. LB232. **0000161904**
6. Chaney, R.L., Reeves, P.G., Kukier, U., Ryan, J.A., Green, C.E. 2004. Food chain transfer and bioavailability of Cd and other elements in plants grown on biosolids amended soils [abstract]. In: Proceedings of the Sustainable Land **0000152863**

Project Number: 5450-51000-035-00D **Accession:** 0407991 **FY:** 2004

Application Conference, Buena Vista, Florida, January 4-8, 2004. p. 22.

7. Scheett, A.J., Johnson, L.K., Hunt, J.R. 2003. Algorithms predicting dietary iron bioavailability: comparisons with measurements from controlled, whole diets [abstract]. Joint 5th International Food Database Conference and 27th National Nutrient Databank Conference, June 30 to July 3, 2003, Washington, D.C. E51:75. **0000145612**

Approved: ROOS ERIC E **Date:** 09/14/2004

Project Number: 5450-51000-035-04T **Accession:** 0402795 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 02/22/2000 **Term Date:** 02/21/2005

National Programs: 107 100% Human Nutrition

Title: HEALTH BENEFITS OF FOOD FORMS OF SELENIUM

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-3K95-0-0813

Organization Name: GENERAL MILLS

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
A human feeding study with high-Se wheat breakfast cereal, planned in 2002, and initiated in 2003, was completed. Twenty-four healthy volunteers consumed for three months whole wheat cereal made from normal wheat or selenium enriched wheat. Primary endpoints of the study were effects of Se on exercise-induced oxidative stress and the effects of whole wheat consumption on production of volatile fatty acids (from fermentation). The fermentation and volatile fatty acid and effects of exercise on oxidative stress analyses are complete and data are being statistically analyzed. Blood, urine and fecal samples collected during the study are in the process of being analyzed.

B. Other significant accomplishments:
A manuscript detailing the results of a study of the benefits of selenium from wheat on measures of the inflammatory response was prepared and submitted for publication. We have received the initial reviewer comments and the manuscript is now being revised accordingly.

C. Special populations:
None

D. Progress Report

This report serves to document research conducted under 58-3K95-0-0813 Cooperative Research and Development Agreement between ARS and General Mills, Inc. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The purpose of this CRADA is to study whether high-Se wheat from high-Se areas of the Northern Great Plains that has been converted into a whole wheat cereal product can be used to safely and effectively provide supplemental Se to humans with adequate Se status.

The ARS is conducting human and animal studies and analyzing all samples collected during the experiment. General Mills, Inc. is converting high-Se wheat into a ready-to-eat cereal product.

Publications:

Project Number: 5450-51000-035-04T **Accession:** 0402795 **FY:** 2004

None

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51000-035-05T **Accession:** 0403407 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 09/01/2000 **Term Date:** 08/31/2004

National Programs: 107 100% Human Nutrition

Title: HIGH SELENIUM MEAT, WHEAT, AND BROCCOLI: A MARKETABLE ASSET?

Period Covered From: 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 01-5450-3-0157

Organization Name: INITIATIVE FOR FUTURE AGRICULTURE AND FOOD SYSTEMS (IFAFS)DEPARTMENT OF AGRICULTURE, CSREES

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment:

Broccoli consumption is associated with decreased cancer incidence and selenium enhancement of broccoli further increases its anti-cancer potential. However, it is not known whether selenium enrichment by Se fertilization affects other nutritive qualities of broccoli. A series of studies demonstrated that selenium enrichment is associated with a dramatic decline in glucosinolate production - glucosinolates are secondary plant compounds that may be responsible for the anticarcinogenic properties of unenriched broccoli. Selenium enrichment also decreased the phenolic content of broccoli - especially of the cinnamic acid derivatives. These results demonstrate that it may not be possible to simultaneously maximize all bioactive components of a single food - enhancement of one component may result in a simultaneous decrease in other bioactive compounds.

B. Other Accomplishments:

The mechanism by which selenium from high selenium broccoli inhibits cancer is not known, but reduction of oxidative stress is a possible mechanism. A study in mouse hepatoma cells evaluated the ability of various broccoli extracts to inhibit hydrogen peroxide-induced DNA strand breaks (a measure of protection against oxidative stress). Unenriched broccoli inhibited approximately 40% of strand breaks whereas modest selenium enrichment of broccoli inhibited 100% of strand breaks. These results demonstrate that some of the anti-carcinogenic activity of broccoli may be a result of inhibition of oxidative stress. They also demonstrate that selenium enrichment of broccoli greatly increases its anticarcinogenic potential.

Nutrient databases, such as the one maintained by the USDA, give the mineral content of many foods, and these database values often are utilized for designing diets. A human feeding study necessitated determining the selenium concentration of many foods, and the determined values were compared to USDA database values. For a general class of foods, such as pasta, average determined values were in agreement with database values, but values for a specific brand often differed from the database by several orders of magnitude. These results demonstrate that nutrient databases are useful for general assessment of nutrient intakes of populations, but are not reliable for formulation of specific diets.

Project Number: 5450-51000-035-05T **Accession:** 0403407 **FY:** 2004

C. Special populations:
None

D. Progress Report:

This report serves to document research conducted under 1-5450-3-0157 Trust Fund Cooperative Agreement between ARS and the Initiative for Future Agricultural and Food Systems, CREES/USDA. In addition to work conducted at the GFHNRC, money from this grant has been transferred to the following projects: 5450-51000-035: -06S, -07G, -08S, -09S, -010G, -11S. For complete details of work accomplished under this project, annual reports detailing work conducted in the above agreements also should be consulted. Additional details of this research can be found in the report for the parent CRIS 5450-51000-035-00D.

Scientific Publications: **Log 115:**

1. Finley, J.W., Grusak, M.A., Keck, A., Gregoire, B.R. 2004. Bioavailability of selenium from meat and broccoli as determined by retention and distribution of se75. Biological Trace Element Research. 99:191-209. **0000147859**
2. Finley, J.W. 2003. Reduction of Cancer Risk by Consumption of Selenium-Enriched Plants: Enrichment of Broccoli with Selenium Increases the Anticarcinogenic Properties of Broccoli. Journal of Medicinal Food. 6(1):19-26. **0000142908**
3. Keck, A.S., Finley, J.W. 2004. Cruciferous Vegetables: Cancer Protective Mechanisms of Glucosinolate Hydrolysis Products and Selenium. Integrative Cancer Therapies. 3(1):5-12. **0000153970**
4. Garvin, D.F., Finley, J.W., Carver, B.F. 2003. Genotypic and environmental effects on seed selenium content in common wheat [abstract]. ASA-CSSA-SSSA Annual Meeting Abstracts. CD-ROM. Paper No. 620. **0000151979**

Approved: ROOS ERIC E **Date:** 08/12/2004

Project Number: 5450-51000-035-06S **Accession:** 0404307 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 03/01/2001 **Term Date:** 09/15/2005

National Programs: 107 100% Human Nutrition

Title: AGRICULTURAL PRODUCTION ASPECTS OF HIGH SELENIUM MEAT AND WHEAT

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-1-0310

Organization Name: NORTH DAKOTA STATE UNIVERSITY

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2002:
Selenium consumed in amounts greater than the dietary recommendation reduces cancer risk and incidence. During pregnancy a number of tissues, including the fetus, are fast growing, it is not known if high dietary intakes of selenium also inhibit growth of these tissues. Rats at three physiological stages - non-pregnant, gestating and lactating, were fed supra nutritional concentrations of dietary selenium (2 mcg/g diet) for 21 days with the selenium supplied as highly bioavailable selenomethionine, or less bioavailable selenocysteine. Selenium from selenomethionine accumulated to a much greater extent than did selenium from selenocysteine. Despite differences in accumulation, the chemical form of selenium had no impact on any measure of pregnancy, gestation or fetal health. These data will be important in helping determine whether selenium supplementation poses a potential risk to pregnant women.

B. Other Accomplishments:
Beef is the greatest source of selenium in the North American diet, and feeding beef cattle high-selenium feedstuffs increases the selenium content of muscle tissue; previous studies by this group demonstrated that selenium-enriched beef could be produced by feeding high-selenium feedstuffs for a relatively short amount of time, but the effect of this on the quality of beef has not been evaluated. A comprehensive study demonstrated that selenium enhancement did not affect taste or other organo-sensory aspects, but selenium enhancement did slightly extend shelf life of retail cuts. These results demonstrate that increasing the selenium concentration of beef does not adversely affect consumer appeal of retail cuts of beef.

C. Special populations:
None

D. Progress Report:

This report serves to document research conducted under a reimbursable agreement between ARS and the Department of Animal Science, North Dakota State University. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

Project Number: 5450-51000-035-06S **Accession:** 0404307 **FY:** 2004

Dr. J. Caton was a co-author on the grant that has supplied the money for this project. The overall project is doing a comprehensive study of how high-Se wheat, meat and broccoli are produced and utilized by animals. Dr. Caton is conducting the Animal Science portion of the project.

Scientific Publications: **Log 115:**

1. Lawler, T.L., Taylor, J.B., Finley, J.W., Caton, J.S. Effect of feeds naturally high in selenium on performance and selenium concentration in various tissues of finishing beef steer. Journal of Animal Science. 2003. v. 81 (Suppl.1) Abstract p. 80. **0000149382**
2. WSASAS Proc. 53:534-36, 2003. **0000149542**
3. Soto-Navarro, S.A., Williams, G.J., Taylor, J.B., Finley, J.W., Caton, J.S.2003. Effects of selenium source of characteristics of selenium digestibility in finishing beef steers [abstract]. Journal of Animal Science. 81(Suppl.2):95. **0000159748**
4. Lawler, T.J., Taylor, J.B., Grings, E.E., Finley, J.W., Caton, J. 2003. Selenium concentration and distribution in range forages from four locations in the northern great plains. Research Update for Fort Keogh Livestock and Range Research Laboratory p. 82. 2003. **0000167588**
5. Soto-Navarro, S.A., T.L. Lawler, J.B. Taylor, L.P. Reynolds, J.J. Reed, J.W. Finley, and J.S. Caton. 2004. Effect of high selenium wheat on visceral organ mass, and intestinal cellularity and vascularity in finishing beef steers. J.Anim. Sci. 82:1788-1792. **0000156055**
6. Lawler, T.L., J.B. Taylor, d, J.W. Finley, and J.S. Caton. 2004. Effect of Supranutritional and Organically-bound Selenium on Perfomance, Carcass Characteristics, and Selenium Distribution in Finishing Steers. J.Anim. Sci. 82:1488-1493. **0000156056**

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51000-035-09S **Accession:** 0404746 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 06/01/2001 **Term Date:** 05/31/2006

National Programs: 107 100% Human Nutrition

Title: HEALTH BENEFITS OF INTERACTING PHYTOCHEMICALS IN BROCCOLI

Period Covered From: 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-1-0330

Organization Name: UNIV OF ILLINOIS

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment of 2002:

Broccoli consumption is associated with decreased risk of many cancers, and consumption of selenium enriched broccoli is associated with protection against chemically-induced carcinogenesis in rats. The in vivo effects of broccoli and selenium-enriched broccoli consumption by humans are unknown. A study fed volunteers either 9 or 90 grams of broccoli per day for two months. Additionally, broccoli was either enriched or unenriched in selenium. Subjects resided in a metabolic ward for portions of the study in order to collect feces and urine and conduct neuro-psychological experiments. Primary endpoints of the study were activity of Phase II proteins, selenium status, selenoprotein activity and sulforaphane elimination in the urine. Additionally DNA strand breaks were measured in the leucocytes of all subjects. The study is now complete and samples are in the process of being analyzed.

D. Progress Report:

This report serves to document research conducted under 58-5450-1-0330 Specific Cooperative Agreement between ARS and the Department of Chemistry, University of Illinois. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

Dr. E. Jeffery at the University of Illinois has an active research program examining the health benefits of glucosinolate compounds found in broccoli. She has collaborated with a plant breeder to develop different strains of broccoli with different concentrations of glucosinolates. Our work with broccoli has been examining the health benefits of selenium that accumulates in broccoli grown under special high-Se conditions. This project is designed to join these two lines of research and determine whether the two compounds have any nutritional interaction.

Scientific Publications: **Log 115:**

1. Hintze, K.J., Wald, K., Jeffery, E.H., Finley, J.W., Zeng, H. 2003. Thioredoxin reductase in human hepatoma cells is transcriptionally regulated by sulforaphane and other electrophiles via an antioxidant response element. Journal of Nutrition. 133:2721-2727. 0000167640

Project Number: 5450-51000-035-09S **Accession:** 0404746 **FY:** 2004

Approved: ROOS ERIC E **Date:** 08/05/2004

Project Number: 5450-51000-035-10G **Accession:** 0404556 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 06/07/2001 **Term Date:** 06/06/2005

National Programs: 107 100% Human Nutrition

Title: USE OF GEOLOGICAL INFORMATION SYSTEM TO VISUALIZE HIGH SELENIUM SOIL AND CROPS

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 59-5450-1-0325

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**
A. Single most significant accomplishment of 2004:
Because selenium may reduce cancer, many agricultural producers wish to increase the selenium concentration of their crops. Recommendations of the best way to accomplish this require a good understanding of the relationship between selenium in the soil and selenium accumulation in plants. This was studied on a ranch in a location known to have high concentrations of Se in the soil in Central South Dakota from May 2001 until July of 2003. Multiple water, soil and plant samples were collected in various locations (on the ranch) across the 2001-2002 growing seasons, and mineral concentrations and soil chemistry analyses were conducted. Only a small percent of total selenium was water soluble, and total and water soluble selenium were highly correlated to sodium sulfate and total sulfur concentrations, likewise organic carbon was positively related to selenium concentrations. It has long been recognized that plants grown on soils with the same total selenium content often accumulate greatly different amounts of selenium. These results serve to elucidate some of the relationships that may affect selenium accumulation in plants, and are useful for developing agricultural production methods that allow for consistent accumulation of high concentrations of selenium in plants.

B. Other accomplishments:
None
C. Special populations:
None

D. Progress Report:
This report serves to document research conducted under 59-5450-1-0325 Grant Agreement between ARS and the Department of Geology, University of North Dakota. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The purpose of this project is to examine the geological, geographical and soil-related factors that result in the development of high-Se soils and the subsequent production of high-Se crops. Knowledge of these factors is essential if producers ever attempt to market high-Se wheat on a regular basis.

Publications:

Project Number: 5450-51000-035-10G **Accession:** 0404556 **FY:** 2004

None

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/29/2004

Project Number: 5450-51000-035-11S **Accession:** 0404830 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 09/01/2001 **Term Date:** 08/31/2006

National Programs: 107 100% Human Nutrition

Title: SELENIUM SUPPLEMENTATION OF SUBJECTS WITH EXTREMELY LOW SELENIUM INTAKES

Period Covered From: 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-1-0163F

Organization Name: INST OF NUTRITION & FOOD HYGIENE

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2004:
Dietary selenium may have different metabolic fates depending on the selenium status of the individual. Subjects in North America that are in adequate selenium status consume supplemental selenium primarily for its cancer-protective effects, whereas people that are selenium deficient consume supplemental selenium for restoring the basic biochemical functions of selenium such as selenoprotein activity. Subjects living in the mountains of south-central China are among the most selenium-deficient in the world; a supplementation study was conducted there to determine the bioavailability of selenium from meat, wheat and broccoli. High-Se beef, wheat and broccoli were cooked, freeze-dried and vacuum-packaged into individual servings containing a daily dose of supplemental Se. Sixty healthy men in DaChang County, Sichuan Province, were fed these supplements for three months and blood and urine were collected. Duplicate samples have been shipped to the Beijing Academy of Preventive Medicine, as well as to the Grand Forks Human Nutrition Research Center, where all are now undergoing analyses. Results of this study will demonstrate whether selenium-enhanced foods are also effective as selenium supplements for humans that are severely selenium deficient.

B. Other Accomplishments:
Selenium accumulates in the brain and limited data suggests that supplemental selenium improves neuropsychological function. As part of the feeding study conducted in DaChang, China, subjects were administered a battery of cognitive, neurological and psychological tests. Tests are now being analyzed and results will be used to determine whether selenium supplementation of deficient individuals is necessary for optimal brain function.

C. Special populations:
None

D. Progress Report:
This report serves to document research conducted under 58-5450-1-F163 Specific Cooperative Agreement between ARS and the Chinese Academy of Preventive Medicine. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

An overall objective in our laboratory is to determine whether foods enriched in Se

Project Number: 5450-51000-035-11S **Accession:** 0404830 **FY:** 2004

can safely and effectively be utilized as supplemental sources of Se. To this end we are conducting field studies that are examining the geological and biological processes that cause accumulation of Se in soil, plants and animals. We also are conducting basic nutritional trials in animals that are determining whether Se from wheat, meat and broccoli is effective for a number of health-related problems such as prevention of colon cancer. The ultimate measure of the "usefulness" of these food sources of Se is to feed them to a target population and measure basic aspects of health and metabolism.

This project is feeding high-Se broccoli, wheat and meat to healthy humans living in a low-Se area of China. We have chosen to study this area of China because Se intakes there are the lowest in the world. This is a cooperative project with the Chinese Academy of Preventive Medicine.

Publications:

None

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/29/2004

Project Number: 5450-51000-035-13T **Accession:** 0405631 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 07/01/2002 **Term Date:** 06/30/2005

National Programs: 107 100% Human Nutrition

Title: COMBATING IRON DEFICIENCY: ABSORPTION & EFFICACY IN HUMANS OF ELEMENTAL IRON POWDERS & HEME IRON

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 03-5450-3-0237

Organization Name: COOPERATIVE STATE RESEARCH EDUCATION & EXTENSION SERVICE (CSREES), U.S. DEPARTMENT OF AGRICULTURE

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. None
B. None
C. None
D. This report serves to document research conducted under 3-5450-3-0237 Trust Agreement. Additional details can be found in the report for the parent CRIS 5450-51000-035-00D (formerly 5450-51000-032-00D), concerning the USDA CSREES Nutritional Research Initiative competitive grant 2002-01885 (and tracked by CREES as CRIS 0192629) entitled "Combating Iron Deficiency: Absorption and Efficacy in Humans of Elemental Iron Powders and Heme Iron". This project has two objectives: 1) to determine the efficacy of fortifying food with elemental iron powders, relative to equivalent amounts of ferrous sulfate, or of supplementing with a limited amount of iron in the heme form, on serum ferritin in women with low iron stores, and 2) to determine the absorption of irradiated electrolytic iron powder, relative to ferrous sulfate, as affected by dose and by interactions with ascorbic acid and phytic acid. This year we report the results of the first objective. In a randomized, blinded, controlled efficacy trial, 52 premenopausal women with moderate to low iron stores received: a) placebo; b) 5 mg iron as heme iron (VitaHeme, USA); or 50 mg iron as c) electrolytic iron (A-131, USA); d) reduced iron (ATOMET 95SP, Canada); or e) bakery-grade FeSO4 ($FeSO4 \cdot H2O$, USA). The heme iron was given in 2 capsules/d and the other sources in 3 wheat rolls/d, provided for 12 wk. The change in body iron was assessed from the serum transferrin receptor/serum ferritin ratio (JD Cook. Blood 101:3359, 2003), which detected the treatment differences more sensitively than several other indexes of iron status. Body iron (mg/kg body wt) increased with all four iron sources (LSM±SEM): FeSO4 (2.0 ± 0.5, $p<0.004$), electrolytic (1.7 ± 0.5, $p<0.008$), reduced (1.0 ± 0.4, $p<0.03$), and heme (1.0 ± 0.4, $p<0.04$), but not with placebo (0.1 ± 0.3, NS). The results indicate that the reduced and electrolytic iron sources were approximately 50 and 85% as effective as FeSO4 and that 5 mg iron in the heme form was half as effective as 50 mg of iron from FeSO4 for improving body iron in humans. Because the reduced and electrolytic iron sources are more stable and can extend the shelf-life with fewer adverse organoleptic changes in fortified foods, in comparison with FeSO4, these results can be useful in selecting forms and amounts of iron to use for food fortification.
Additional data analysis and manuscript preparation is in progress.

Project Number: 5450-51000-035-13T **Accession:** 0405631 **FY:** 2004

Scientific Publications: **Log 115:**

1. Swain, J.H., Johnson, L.K., Hunt, J.R. 2004. Combating iron deficiency: bioavailability of iron from two elemental iron powders and a heme iron supplement in humans [abstract]. Federation of American Societies for Experimental Biology Journal. 18:A155. 0000156303

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51000-035-14T **Accession:** 0405700 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 06/01/2002 **Term Date:** 05/31/2005

National Programs: 107 100% Human Nutrition

Title: CHANGING THE NUTRITIONAL COMPOSITION OF BEEF: CAN WE MITIGATE THE RISK OF COLON CANCER?

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-2-0421

Organization Name: NATIONAL CATTLEMEN'S BEEF ASSOCIATION

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2003:
Beef raised on selenium-rich pastures or forages may contain selenium in concentrations of an order of magnitude greater than average (beef selenium concentrations). Such beef may be an excellent source of selenium for individuals wishing to supplement their diet, however the bioavailability of selenium from high-selenium beef is not known; a human study was designed and is being conducted to determine selenium bioavailability from selenium-enhanced beef. Human subjects selected for blood selenium concentrations below the national average were fed controlled diets that contained a modest portion of beef (100 grams) that was either low in selenium or selenium-enriched. There were three dietary treatments: High selenium beef, average selenium intake of ~ 300 micrograms selenium/day; low selenium beef with an average selenium intake of ~ 40 micrograms/day; and low selenium beef plus supplemental selenomethionine, with an average selenium intake of ~ 300 micrograms per day. Subjects were fed diets for 15 weeks and the major endpoints of the study were retention of a stable isotope of selenium, selenium status, antioxidant status and gene activation (assessed by a gene micro array). The study is in progress and approximately one-fourth of the subjects will complete the study in August. Additional groups are starting at regular intervals throughout the summer and fall and the study should be complete before 2005. Results of the study will be important for evaluating whether selenium in beef is bioavailable and whether selenium-enhanced beef is a useful method for supplementing the diet with selenium.

B. Other significant accomplishments:
None

C. Special populations:
None

D. Progress Report
This report serves to document research conducted under 58-5450-2-0421 Trust Agreement between ARS and the National Cattlemen's Beef Association. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

Project Number: 5450-51000-035-14T **Accession:** 0405700 **FY:** 2004

The purpose of this Trust is to study whether high-Se beef produced in high-Se areas of the Northern Great Plains can be used to safely and effectively provide supplemental Se to humans with adequate Se status. Animal studies will determine if Se from high-Se beef is effective for prevention of colon cancer.

Publications:

None

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51000-035-16S **Accession:** 0405998 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 09/16/2002 **Term Date:** 09/15/2007

National Programs: 107 100% Human Nutrition

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS TO SUPPORT RESEARCH ON HUMAN MINERAL NUTRITION

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-2-0335

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. None
B. None
C. None
D. This report serves to document research conducted under 58-5450-2-0335 Specific Cooperative Agreement between ARS and University of North Dakota Physics Department (Dr. Glenn Lykken, Health Physicist). Additional details of the research can be found in the reports for the parent CRIS projects 5450-51000-032-00D and 5450-51000-035-00D and related CRIS project 5450-51000-034-00D.

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine whole body retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true "tracer" that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, applying an interdisciplinary approach to answering nutrition questions with whole body counting methodology.

Accomplishments this year included health physicist support of investigations to assess bioavailability of calcium, iron, and zinc using gamma-emitting radiotracers in human absorption studies. The health physicist led the work of the FDA-sanctioned UND Radioactive Drug Research Committee, which reviewed and provided oversight for all nutrition experiments employing radioactive tracers.

This year, in an assessment of human bismuth-214 (a decay product of radon) retention, individual detector data revealed the need to modify the shape and size of phantoms used to produce standards for radionuclide tracer measurements. Phantoms currently in use tend to underestimate radionuclide concentrations in the head and overestimate them in the lower torso and legs. A more realistic and economical phantom will be developed and tested. It was also determined that a 5 cm x 5 cm

Project Number: 5450-51000-035-16S **Accession:** 0405998 **FY:** 2004

cylindrical detector shape functioned better than a 7.5 cm x 7.5 cm cylindrical gamma well detector for collecting Ca-47 data from the calcaneus (heel) when studying regional uptake of calcium in metabolism studies.

Factors affecting calcium uptake and retention in bones were studied in a collaborative experiment with Dr L. Klevay. Two strains of mice, one susceptible to both atherosclerosis and osteoporosis and one normal, were fed atherogenic diets (see Parhami et al, J Bone Min Res 16:182, 2001). Under a Research Reactor Sharing Grant [Project RS03-010, Research Reactor Center, University of Missouri-Columbia, (MURR)] calcium enriched with 46Ca was delivered to the MURR and activated into 47Ca. A total of 100 µCi was produced and 1 µCi was fed to each of 60 mice whose femurs were analyzed for both 47Ca and 47Sc gamma emissions with a high-purity germanium (HpGe) detector. Ca-47 and 47Sc gamma emissions were greatest in the genetically susceptible mice. Scandium-47 gamma emissions, in the absence of 47Ca gamma emissions, from femur muscles were detected. Apparently 47Sc, produced in the decay of 47Ca, was taken up by muscle. The muscle 47Sc decayed with a 3.4 d half-life as compared to a longer effective half-life in the presence of its parent 47Ca, suggesting that the 47Sc was present in the muscle in the absence of detectable/appreciable 47Ca. The results indicate greater calcium retention by these mice genetically susceptible to atherosclerosis and osteoporosis.

An evaluation of over 15 years of UND Radon Monitoring Facility data indicated that radon concentrations in Grand Forks homes paralleled the body activity of bismuth-214 (a decay product of radon) in community volunteers. These activities were greatest in the fall quarter and least in the spring quarter. These seasonal variations are consistent with increased body radon accumulation in enclosed buildings, and possibly increased body fat (sites of physiological radon storage) during winter months.

The sensitive detection of gamma-emitting isotopic tracers, provides uniquely sensitive measurements of mineral retention in humans and animal models, as affected by nutritional status, dietary sources of nutrients, and genotype.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/29/2004

Project Number: 5450-51000-035-17T **Accession:** 0406927 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 01/01/2003 **Term Date:** 12/31/2007

National Programs: 107 100% Human Nutrition

Title: NUTRITIONAL VALUE OF BUCKWHEAT: TRACE ELEMENT VARIABILITY AND BIOAVAILIBILITY AND FAGOPYRITOL CONTENT

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0406

Organization Name: MINN-DAK GROWERS, LTD.

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2003:
North Dakota produces the majority of buckwheat grown in the US and much of that is grown in areas known to have high-selenium soils. A survey of buckwheat grown in these areas has shown it to contain between 0.5 and 1.0 micrograms selenium per gram, making it a nutritionally important source of selenium. However, the bioavailability of selenium from buckwheat is not known. To determine the bioavailability of selenium from buckwheat we repleted the selenium status of selenium-deficient rats with buckwheat enriched in selenium. Other rats were repleted with the pure chemical forms of selenium, selenomethionine and selenite. After 49 days of selenium repletion, rats were killed and selenium status was determined by measuring selenoprotein activities and selenium concentrations in important tissues. Initial analyses (analyses are ongoing) showed that selenium from buckwheat was only 45-60% as bioavailable as selenomethionine and selenite. However, the average concentration of selenium in North Dakota buckwheat is almost twice the average concentration in US cereal grains, thus selenium-enriched buckwheat is an important dietary source of selenium, despite slightly lower bioavailability.

B. Other significant accomplishments:
Buckwheat may be an excellent source of several nutritionally important chemicals, including trace elements such as selenium, but sources of variation in the trace element content of field grown buckwheat have not been characterized. A study to determine the variability of minerals, with an emphasis on selenium, that began in 2002 was repeated in the summer of 2003. Briefly, multiple samples of buckwheat were collected at the time of harvest at multiple sites across North Dakota (collaborative with MinnDak Growers, Ltd., Grand Forks, ND); samples were analyzed for trace minerals including selenium. Two years of analyses now show that all North Dakota buckwheat samples are average to high in selenium (compared to other grains), but concentrations are variable and affected by geographic location. The Geographic Information Service (GIS) database is being used to develop models that predict environmental and production variables that result in selenium enhancement of buckwheat.

C. Special populations:
None

Project Number: 5450-51000-035-17T **Accession:** 0406927 **FY:** 2004

D. Progress Report
This report serves to document research conducted under 58-5450-3-0406 Trust Fund Cooperative Agreement between ARS and MinnDak Growers, Ltd. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The purpose of this agreement is to study the nutritional value of buckwheat, with emphasis on buckwheat as a source of nutritionally essential minerals. MinnDak Growers, Ltd. is collecting buckwheat and soil samples from buckwheat producers, and preparing those samples for analysis. The GFHNRC is conducting all chemical analyses, and is planning and conducting studies in rats to determine the bioavailability of minerals from buckwheat.

Publications:
None

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/29/2004

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 09/29/2003 **Term Date:** 09/28/2008

National Programs: 107 100% Human Nutrition

Title: HUMAN STUDIES RESEARCH

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 58-5450-3-0324

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

This report serves to document research conducted under Specific Cooperative Agreement #58-5450-3-324 between ARS and University of North Dakota (UND). Additional details of the research, including specific research accomplishments, can be found in the reports for the CRIS projects 5450-51000-033-00D (formerly 5450-51000-038-00D), 5450-51000-034-00D, 5450-51530-009-00D (formerly 5450-51530-008-00D), 5450-51000-031-00D, 5450-51000-035-00D (formerly 5450-51000-032-00D).

To improve the nation's nutrition and health (Goal 4 of the Agricultural Research Service Strategic Plan, 2003-2007), research is necessary to generate new knowledge in human nutrition, to improve the understanding of optimal nutrient requirements for known and new classes of nutrients at all stages of the life cycle, and to better understand relationships between diet and health. Although much can be learned using basic biochemical, cellular, and animal models of human nutrition, some questions require direct human studies to assure that the results are applicable to humans.

This agreement between ARS and UND is based on a mutual interest in human nutrition as it pertains to the maintenance of optimum health, including reduction of disease risk. The objective of the agreement is to investigate the role of nutrients in human health, to determine their bioavailability from foods and mixed diets, to investigate their biological activities in cancer prevention, in bone and joint health, in cardiovascular health, and in physiological and psychological development and function. Diets designed to contain known amounts of essential and non-essential nutrients, or foods containing specific nutrients or other bioactive components, are provided to human volunteers under controlled conditions of dietary intake, so that their effects on clinical chemical (blood constituents), physiological (blood pressure, cardiovascular function respiratory function, neuro-muscular function), neurological (mood, neurologic function), urinary and fecal excretion, and other measures of biological activity and health status may be determined. Human studies include residential, non-residential and field-based investigations using such approaches as dietary recall, metabolic balance, radio/stable isotope retention, physiological/neurological function assessment, and specific metabolic/enzyme analyses. Glenn Lykken (UND Department of Physics) and Jeffrey Holm (UND Department of Psychology) are the principal investigators. UND provides an Institutional Review Board to review all protocols for studies involving human subjects at the Grand Forks Human Nutrition Research Center, provides the competent personnel to participate in the planning and execution of studies,

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2004

collaborates with ARS on related animal model studies, and provides peer review of scientific manuscripts.

This research is directly related to five components of the ARS National Human Nutrition 107 research plan: (1) Human Nutrition Requirements, (2) Diet, Genetics, Lifestyle, and the Prevention of Obesity and Disease, (5) Health Promoting Intervention Strategies for Targeted Populations (6) Health Promoting Properties of Plant and Animal Foods, and (7) Bioavailability of Nutrients and Food Components. The research provides knowledge to policy makers and other scientists for setting guidelines to improve human nutrition and reduce disease risk.

2. **List the milestones (indicators of progress) from your Project Plan.**

This specific cooperative agreement provides support for human studies. Specific milestones are documented in the CRIS projects cited above. In support of those milestones, this report addresses milestones for conducting key GFHNRC human studies after approval by the UND IRB.

Year 1 (FY2004)

Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
Study 006 Human absorption of iron fortification sources assessed using an isotope displacement method
Study 007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?
Study 049 Body iron excretion
Study 144 Effects of low, moderate and high zinc intakes on copper nutriture
Study 403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
Complete data collection for approved studies
Study 064 Meat protein and calcium: Do they interact synergistically or antagonistically?
Study 098 Effect of daily intake of wheat cereal containing various concentrations of selenium on selenium status in humans
Study 501 Improved health in humans consuming broccoli high in sulforaphane and selenium
Study 404 Physical health, fitness, nutrition, and mental health in Northern Plains Indians

Year 2 (FY2005)

Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
Study 006 Human absorption of iron fortification sources assessed using an isotope displacement method
Study 049 Body iron excretion
Study 403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
Complete data collection for approved studies
Study 007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?
Study 090 Determining dietary zinc requirements from adaptation in zinc absorption
Study 144 Effects of low, moderate and high zinc intakes on copper nutriture

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2004

Year 3 (FY2006)
Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
Study 049 Body iron excretion
Study 403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?
Complete data collection for approved studies
Study 006 Human absorption of iron fortification sources assessed using an isotope displacement method

Year 4 (FY2007)
Collaborate in planning and approvals for human studies
Recruit subjects and begin or continue data collection for approved studies
Complete data collection for approved studies
Study 049 Body iron excretion
Study 403 Is supplementation with calcium plus trace minerals superior to calcium alone in attenuating bone loss in healthy postmenopausal women?

3. **Milestones:**

A. List the milestones that were scheduled to be addressed in FY 2004. How many milestones did you fully or substantially meet in FY 2004 and indicate which ones were not fully or substantially met, briefly explain why not, and your plans to do so.

Collaborate in planning and approvals for human studies. In 2004, approvals were obtained for:
Study 006 Human absorption of iron fortification sources assessed using an isotope displacement method
Study 007 Beef as a component of a healthy diet: does it improve selenium and trace element status of healthy men and women?
Study 090 Determining dietary zinc requirements from adaptation in zinc absorption
Study 145 Does magnesium deficiency induce an excitotoxicity detrimental to bone health?

Recruit subjects and begin or continue data collection for studies. In 2004, this was accomplished for all projects indicated in the milestone.

Complete data collection for studies. In 2004, this was accomplished for all projects indicated in the milestone.

B. List the milestones that you expect to address over the next 3 years (FY 2005, 2006, & 2007). What do you expect to accomplish, year by year, over the next 3 years under each milestone?

As delineated in item 2 above, each year, human studies initiated as part of project plans for the CRIS projects cited above, or as part of related, externally-funded projects will be collaboratively planned, approved, and scheduled for recruitment and data collection. We expect to accomplish all milestones addressed above, and include additional milestones in the latter years, as new projects are approved through the UND Institutional Review Board.

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2004

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment during FY 2004 (one per Research Project): The study (#064) "Meat protein and calcium: Do they interact synergistically or antagonistically?" was completed. After review of 241 applications, 36 postmenopausal women participated in this study that controlled their diets for several weeks and measured calcium absorption using a radiotracer. A high meat diet improved calcium retention in these postmenopausal women when calcium intake was low, but had no minimal when calcium intake was high. See additional information in the report for CRIS 5450-51000-034-00D.

B. Other Significant Accomplishment(s), if any.

Major accomplishments are also described in the reports for the CRIS projects listed above, and include:
- Completion of data analysis for a study showing that ferrous sulfate, reduced, electrolytic, and heme iron can all improve body iron status in premenopausal women. See additional information in the report for CRIS 5450-51000-032-00D.
- The development of trust and respect among the American Indian tribes in North Dakota that resulted in the completion of survey of Native American health, nutrition and physical activity and preparation of a report summarizing the findings that is being distributed to participating Indian tribes and communities. See additional information in the report for CRIS 5450-51530-009-00D.

C. Significant activities that support special target populations.
A partnership between American Indians, tribal nations, and the USDA, ARS was successfully developed, enabling research on preventive measures to reduce health problems such as obesity and diabetes among American Indians in the Northern Plains (see 5450-51530-009-00D).

D. Progress Report opportunity to submit additional programmatic information to your Area Office and NPS (optional for all in-house ("D") projects and the projects listed in Appendix A; mandatory for all other subordinate projects).

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
This is the first year of this specific cooperative agreement, continuing many years of successful collaboration between the Grand Forks Human Nutrition Research Center and the University of North Dakota. Recent accomplishments include research that determined the bioavailability of zinc and iron from vegetarian diets, the human ability to adapt heme and nonheme iron to differences in dietary iron bioavailability, a normal iron absorption by genetic carriers (~10% of US population) of a mutation associated with hemochromatosis, the bioavailability of selenium from broccoli, a negligible effect of soy protein (substituted for meat protein) on calcium retention; the influence of supplemental zinc on measures of cognitive function in children from disadvantaged US and Chinese populations, and electrocardiographic changes associated with marginal magnesium intakes.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
The accomplishments are documented in manuscripts in peer reviewed scientific journals, and in presentations at scientific and public meetings. See the reports for the CRIS projects listed above.

Project Number: 5450-51000-035-18S **Accession:** 0407722 **FY:** 2004

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
See the reports for the CRIS projects listed above.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 08/12/2004

Project Number: 5450-51000-036-01R **Accession:** 0406009 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 07/15/2002 **Term Date:** 06/30/2007

National Programs: 107 100% Human Nutrition

Title: DETERMINATION OF NUTRIENT EFFECTS ON CANCER SUSCEPTIBILITY ON EPIGENETIC PROCESSES IN ANIMAL MODELS

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** No
Terminate in Two Months? No

Agreement Number: 02-5450-2-0217

Organization Name: NATIONAL CANCER INSTITUTE, DEPARTMENT OF HEALTH AND HUMAN SERVICES

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

This report serves to document research conducted under an interagency agreement with the National Cancer Institute. The purpose of this agreement is to examine the interactive effects of dietary selenium and folate on cancer susceptibility, global and gene specific DNA methylation, DNA methyltransferase activity, and one-carbon metabolism using animal models. This work supports that done in 5450-51000-031-00D, Role of Selenium on Gene Expression, Cell Cycle and Molecular Mechanisms in Cancer Risk.

This research indicates the possibility that dietary selenium and folic acid prevent cancer-causing genes (oncogenes) from being turned on and/or cancer-suppressing genes (tumor suppressor genes) from being turned off. Both selenium and folic acid can affect the addition of a single carbon molecule (methylation) to specific areas on DNA. The amount and pattern of methylation of DNA can result in genes being turned on or off (and thus affect cancer susceptibility). A preliminary study was conducted to determine the feasibility of using a technique, called RLGS, to scan thousands of genes for differences in DNA methylation in rats fed graded amounts of dietary selenium (as inorganic selenite) and the vitamin folic acid. The results indicate that dietary selenium and folic acid can affect specific DNA methylation patterns. This is important as it provides a mechanism in which diet can affect the cancer process. A large-scale animal experiment is presently underway to repeat and expand this preliminary experiment. Tissues from this experiment will also be used in gene array analysis as another screening tool to determine which genes are affected by selenium and folic acid. The experiment will also test the form of selenium (selenite, selenomethionine, and Se-methylselenocysteine) to determine the best form of selenium for cancer prevention.

We expect that numerous genes, as determined by microarray analysis, will be affected by dietary selenite and/or folic acid. We also expect that several genes will be found to be affected by methylation, as determined by RLGS. Enzyme and metabolite analyses will help us ascertain possible mechanisms or outcome of the differentially methylated gene(s) (and hence reduced or increased expression). The outcome of this research will assist in the elucidation of mechanisms by which specific nutritional factors - selenium and folate - influence DNA methylation, or other novel processes, as well as increase our understanding of these processes and dietary factors in relation to cancer prevention. It is expected that the results will confirm and expand our previous findings that indicted that selenium supplemented at supranutritional concentrations is beneficial when folic acid is

Project Number: 5450-51000-036-01R **Accession:** 0406009 **FY:** 2004

present in the diet but detrimental when folic acid is inadequate.

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/28/2004

FINAL PROGRESS REPORTS

OF

TERMINATED CRIS WORK UNITS

Project Number: 5450-51530-008-00D **Accession:** 0405210 **FY:** 2004
ModeCode: 5450-10-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
NUTRITIONAL DETERMINANTS OF HEALTH
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 02/22/2002 **Term Date:** 04/02/2004

National Programs: 107 100% Human Nutrition

Title: OPTIMAL MINERAL NUTRITION FOR PHYSIOLOGICAL AND PSYCHOLOGICAL DEVELOPMENT, FUNCTION AND HEALTH

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**
Suboptimal dietary intakes of essential micronutrients have been statistically associated with chronic disorders such as obesity, diabetes, cardiovascular disease, depression and dementia. Further, national nutrition surveys indicate that dietary intakes of several essential minerals (e.g., calcium, copper, iron, magnesium, zinc) are less than recommended in many segments of the U.S. population and that mild-to-marginal deficiencies in these and other micronutrients are particularly likely in at-risk and underserved groups (e.g., women, children, elderly, minorities). Unfortunately, controlled studies of the relationships between micronutrients and chronic disease are few and the roles of potential mediating factors such as age, sex, body composition, special diets, lifestyle and genetic factors are poorly understood. For most micronutrients, the potential health benefits and mechanisms of action for physiological (healthy body weight and composition, energy metabolism, brain and cardiac function) and psychological (cognition, emotional and social adjustment, school/work performance) function have not been determined.

This project aims to improve health and enhance quality of life by determining for healthy and at-risk populations micronutrient intakes that optimize physiological and psychological development, function and health. Specific objectives are to: (1) develop new functional bases for establishing mineral element requirements; (2) identify mechanisms of action; and, (3) determine the influence of mediating factors on mineral element requirements. This project is directly related to the following major components of the National Program Action Plan for Human Nutrition (107): Nutrient Requirements; Relationship between Diet, Genetics and Lifestyle and the Prevention of Chronic Disease; and, Health Promoting Intervention Strategies for Targeted Populations. The research addresses priority objectives, including: (1) developing functional markers of mineral intakes and status; (2) identifying mechanisms of action for mineral elements; (3) determining the influence of genetic, environmental and lifestyle factors on mineral requirements; (4) evaluating the interaction of dietary, genetic, environmental and lifestyle factors on obesity and prevention of chronic disease; (5) identifying dietary intervention strategies effective with minority populations; and, (6) characterizing the role of mineral elements in achieving and maintaining optimal physiological and psychological development, function and health.

Controlled studies generate new knowledge to use in making recommendations for dietary intakes that promote optimal development, function and health throughout the life span. Dietary intakes and biochemical indices of mineral status are related to physiologic (e.g., healthy body weight and composition, physical fitness, energy

Project Number: 5450-51530-008-00D **Accession:** 0405210 **FY:** 2004

metabolism, brain and cardiac function) and psychological (e.g., cognition, emotional and social adjustment, school/work performance) measures to determine importance of specific minerals for optimal function and development. A mobile field laboratory, community studies facilities, and a residential metabolic unit are used to conduct survey, supplementation, fortification, and controlled feeding studies with healthy and at-risk groups (e.g., school-aged children, rural elderly, minorities). Randomized controlled trials evaluate the effects of feeding graded dose amounts of minerals, such as iron, zinc, copper and magnesium, in the context of mediating factors (e.g., genotype, controlled stressors). Animal and cell culture studies enhance the efficacy of human studies and help determine the mechanisms of action of functional outcomes.

This project provides experimentally-derived information needed to establish recommendations for dietary intakes of micronutrients throughout the life span and to help American consumers choose foods that optimize physical and mental performance, social and emotional adjustment, and prevent or ameliorate chronic diseases such as obesity, diabetes, depression and dementia. Resulting information is also useful for evaluating food assistance programs, special diets, the efficacy of taking dietary supplements, and the potential benefits of value-added foods to maintain health and well-being. Primary customers for the products of this research are agricultural and commodity groups, the food industry, supplement manufacturers, policy makers, health and nutrition professionals and the general public.

2. **List the milestones (indicators of progress) from your Project Plan.**
This bridging project was a consolidation of prior projects 5450-51000-027-00D, 5450-51000-006-00D and 5450-51000-007-00D, and scheduled for 2 years (02/15/02-4/30/04). This project terminated 04/02/04 and was replaced by project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health. While no formal milestones were established for this project, the following program objectives and anticipated accomplishments were identified.

- Develop functional markers of mineral intakes and status
- Identify mechanisms of action for mineral elements
- Determine the influence of genetic, environmental and lifestyle factors on mineral requirements
- Evaluate the interaction of dietary, genetic, environmental and lifestyle factors on obesity and prevention of chronic disease
- Identify dietary intervention strategies effective with minority populations
- Characterize the role of mineral elements in achieving and maintaining optimal physiological and psychological development, function and health

Specific Anticipated Accomplishments
Year 1 (FY 2003)
- Collect data from second cohort in field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children aged 12-14 years.
Complete data collection in multi-year study to determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, brain electrophysiology, and cardiovascular function of healthy postmenopausal women participating in a controlled metabolic unit study.
- Complete analysis of data from multi-year study to determine the effects of dietary magnesium intakes on neuropeptides associated with brain dysfunction (e.g., mood and sleep disturbances, migraine headaches), and on brain electrophysiology, sleep quantity and quality, and activity levels of healthy postmenopausal women

Project Number: 5450-51530-008-00D **Accession:** 0405210 **FY:** 2004

participating in a controlled metabolic unit study.
Plan community survey to determine in institutionalized and non-institutionalized elderly the relationships among mineral element status, life-style factors, health, body composition, cognitive function, social and emotional adjustment, and daily activities.
- Conduct study to determine the relative benefits for cognitive function of feeding selenium-enhanced meat, wheat and broccoli to adults in the Peoples Republic of China.
- Design and conduct study with rats to determine the effects of dietary zinc and exercise on the induction and expression of zinc-containing enzymes involved in energy production.
- Design and conduct study with rats to determine the effects of feeding graded dose amounts of zinc on in situ skeletal muscle function.
- Design and conduct study with rats to determine the role of iron intake/status in the regulation of energy production by examining uncoupling protein and heat shock protein metabolism.
- Design and conduct study with rats to determine the effects of copper deficiency during pregnancy on the production of reactive oxygen species by brain and muscle mitochondria in neonates.
- Design and conduct study with rats to determine whether nickel and magnesium modify the response to deficient intakes of the other to affect parameters associated with cyclic-GMP signal transduction in the central nervous system resulting in an indication that these two elements are of practical nutritional concern for cognitive and sensory function.
- Design and conduct study with rats to determine whether a high intake of manganese is a practical nutritional concern for brain function, especially when dietary magnesium is.

Year 2 (FY 2004)
- Collect data from third cohort in field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children aged 12-14 years.
- Plan study and begin recruitment of children in grades 4, 7 and 10 to determine the benefit, if any, of fortifying foods with iron and zinc for health, growth, body composition, muscle strength and endurance, psychomotor and cognitive function, school performance and social adaptation.
- Analyze results and report findings of multi-year study to determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, brain electrophysiology, and cardiovascular function of healthy postmenopausal women participating in a controlled metabolic unit study.
- Plan and initiate a survey of institutionalized (assisted living facility) elderly (>70 years) and non-institutionalized spouses/siblings (controls) to determine relationships among mineral element status, life-style factors, health, body composition, cognitive function, social and emotional adjustment, and daily activities.
- Initiate focus groups with Native community leaders and members to plan a health and nutrition survey similar to the NHANES to be conducted with a mobile research laboratory on American Indian reservations in the upper Midwest.
- Collect data and conduct analyses in study to determine the relative benefits for brain function and cognition of feeding selenium-enhanced meat, wheat and broccoli to adults in the Peoples Republic of China.
- Design and conduct study with rats of the effects of feeding graded dose amounts of zinc on skeletal muscle function to test the hypothesis that restricted zinc intake impairs muscle contraction and relaxation, strength and endurance and that the mechanism of action is calcium transport.

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- Design and conduct study with rats to determine the role of iron intake/status in the regulation of energy production by examining uncoupling protein and heat shock protein metabolism.
- Design and conduct study with rats to determine the effects of feeding graded dose amounts of copper on reactive oxygen and nitrogen species on angiogenesis and skeletal muscle apoptosis as beneficial adaptive responses to exercise.
- Design and conduct study with rats to investigate the effects of copper deficiency during pregnancy on the production of reactive oxygen species by brain and muscle mitochondria in neonates.
- Design and conduct study with rats determine whether dietary deprivation of boron impairs sensory functions and brain development and whether impairments are exacerbated by an inadequate intake of long-chain omega-3 fatty acids.

3. Milestones:

This bridging project was a consolidation of prior projects 5450-51000-027-00D, 5450-51000-006-00D and 5450-51000-007-00D, and scheduled for 2 years (02/15/02-4/30/04). This project terminated 04/02/04 and was replaced by project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health. While no formal milestones were established for this bridging project, the following summary indicates how the specific anticipated accomplishments identified for FY 2004 were addressed until the project was terminated.

Fully or Substantially Met

Completed collection of data from third cohort in field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in children aged 12-14 years. Analysis of data from all 3 cohorts to determine need for additional cohorts continues under replacement project.

Initiated contacts with tribal leaders, members and health care providers at White Earth and Turtle Mountain reservations and United Tribes Technical College to plan a health and nutrition survey on American Indian reservations in the upper Midwest. Contacts with representatives from other reservations in North Dakota continue under replacement project.

Collected brain electrophysiology, cognitive performance and mood states data and began analyses to determine the relative benefits for brain function and cognition of feeding selenium-enhanced meat, wheat and broccoli to adults in the Peoples Republic of China. Analyses continue under replacement project.

Designed and conducted study with rats of the effects of feeding graded dose amounts of zinc on in situ skeletal muscle function and found that marginal zinc retards peak force development (strength) and was associated with reduced time to muscle fatigue. Low zinc was also related to decreased carbonic anhydrase activity in the muscles studied.

Designed and conducted study with rats to determine the role of iron intake/status in the regulation of energy production and found that rats fed low dietary iron had a small rate of loss of body temperature (0.08 degrees/hr), not different than the rates determined in the rats fed adequate or supplemental iron. Mitochondrial uncoupling protein, a key regulator of mitochondrial energy production, decreased as well as a specific heat shock protein, only in the rats fed the low iron diet.

Designed and conducted study with rats to determine the effects of feeding graded dose amounts of copper on reactive oxygen and nitrogen species on angiogenesis and skeletal muscle apoptosis as beneficial adaptive responses to exercise. Iron status, shown by increased red cell width distribution, was affected by dietary copper with no evidence of anemia. Extracellular superoxide dismutase

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(SOD3) was found to be a very specific marker in all rats. However, it was a sensitive indicator of dietary copper only in male rats; SOD3 activity was directly related to dietary copper. Among the female rats, SOD3 activity was reduced only in severe copper restriction. Muscle cytochrome c oxidase, another copper-containing enzyme, was a sensitive and specific indicator of dietary copper in all rats and independent of muscle composition (e.g., fast- or slow-twitch).

Designed and conducted study with rats to determine whether dietary deprivation of boron impairs sensory functions and brain development and whether impairments are exacerbated by an inadequate intake of long-chain omega-3 fatty acids. Examination of the eyes of pups by electron microscopic procedures revealed that a diet high in omega-3 fatty acids and boron improved mitochondrial configuration, condition, and abundance of cristae.

Not Fully or Substantially Met

Preliminary planning began for a series of studies to determine the benefit of iron and zinc fortification for health, growth, body composition, muscle strength and endurance, psychomotor and cognitive function, school performance and social adaptation in 4th, 7th and 10th grade children. However, more detailed planning awaits outcome of analyses of current study of zinc fortification of 7th graders. Analysis and reporting of results from multi-year study to determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, brain electrophysiology, and cardiovascular function of healthy postmenopausal women participating in a controlled metabolic unit study awaits recruitment of additional participants to provide sufficient statistical power. Following several years when efforts to recruit individuals to participate in long-term live-in studies were minimally successful, recently revised recruitment efforts appear to be having greater success.

Preliminary planning began for a survey of institutionalized (assisted living facility) elderly (>70 years) and non-institutionalized spouses/siblings (controls) to determine relationships among mineral element status, life-style factors, health, body composition, cognitive function, social and emotional adjustment, and daily activities. Progress towards meeting objective continues under the replacement project.

Prior to termination of this project, we were unable to design and conduct study with rats to investigate the effects of copper deficiency during pregnancy on the production of reactive oxygen species by brain and muscle mitochondria in neonates. This objective will be pursued under the replacement project.

This bridging project was a consolidation of prior projects 5450-51000-027-00D, 5450-51000-006-00D and 5450-51000-007-00D, and scheduled for 2 years (02/15/02-4/30/04). This project terminated 04/02/04 and was replaced by project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health. Milestones for the next 3 years (FY2005-FY2007) are listed below and in the replacement project.

Year 1 (FY 2004-2005)

Plan, obtain institutional and school board approvals, and initiate intervention study to determine relationships among zinc and iron nutrition and cognitive function, psycho-educational performance, body composition and growth in children and adolescents.

Plan, obtain institutional and administrator approvals, and initiate a cross-sectional survey of nutrition, health and function in healthy elderly living in institutionalized and non-institutionalized environments.

Plan, obtain approvals, and initiate a study of the effects of dietary zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity.

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Plan, obtain institutional and reservation approvals, and initiate an observational study to determine the nutrient composition of foods in the food assistance programs and traditional foods on American Indian reservations.

Year 2 (FY 2006)

Conduct study to determine in children and adolescents relationships between zinc and iron nutrition and cognitive function, psychoeducational performance, body composition, growth, and physical fitness in various locations.

Plan and initiate study of dietary magnesium on neurological and psychological functions of postmenopausal women.

Conduct observational study of relationships among zinc, copper and magnesium nutrition and physical and mental health of healthy elderly.

Plan and initiate study of effects of differences in body composition phenotypes of rats on copper and zinc metabolism with increased physical activity in rats.

Analyze samples from study of effects of zinc and copper on mechanisms of adaptation to endurance exercise training among in-bred strains of rats with different phenotypes for running capacity; report results.

Complete the nutrient composition survey of components of food assistance program and include traditional Indian foods.

Plan and initiate study of interaction of dietary boron and essential fatty acids in rats.

Plan weight loss study of obese humans and recruit subjects.

Year 3 (FY 2007)

Conduct study of zinc and iron supplementation in children and adolescents in various locations.

Conduct study of magnesium intake and neurological and psychological functions of postmenopausal women.

Plan and initiate study of copper on adaptation to increased physical activity of out-bred rats with different phenotypes for aerobic capacity.

Develop nutrient database of commodity and traditional Native foods.

Initiate community focus groups to develop appropriate menus including traditional foods to prevent obesity and diabetes in American Indians.

Analyze samples and data from observational study of the elderly; report results.

Conduct weight loss and weight maintenance study.

Plan experimental protocol for study of effects of feeding graded dose amounts of zinc on adaptation to increased physical activity in humans.

4. What were the most significant accomplishments this past year?

This bridging project was a consolidation of prior projects 5450-51000-027-00D, 5450-51000-006-00D and 5450-51000-007-00D, and scheduled for 2 years (02/15/02-4/30/04). This project terminated 04/02/04 and was replaced by project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health.

A. Single Most Significant Accomplishment during FY 2004 (one per Research Project).

Zinc fortification improves memory of young adolescents. Completed data collection from the third cohort of subjects in a multi-year field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in 204 children, aged 12-14 years. Analysis of data from the first two cohorts showed that baseline plasma zinc was inconsistently but significantly related to several measures of attention in girls and to perception and reasoning in boys. Compared to placebo, 20 mg zinc fortification for

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10 weeks improved memory overall (girls: 11% vs 6%; boys: 11% vs 8%) and short-term memory (girls: 13% vs 7%; boys: 11% vs 4%) in particular. Impact: Findings help determine needed zinc intakes to promote optimal physical and behavioral development of peri-pubescent adolescents at-risk for zinc deficiency because of rapid growth.

B. Other Significant Accomplishments.
Abnormal motor function persists following recovery from perinatal copper deficiency. Pregnant rats were fed a low copper diet (0.34 mg/kg) starting on gestation day 7; half also received additional copper in their drinking water (20 mg/L) while the other half drank demineralized water. Pups from all dams were switched to a copper adequate diet (13.2 mg/kg) at weaning and sampled biochemically after 3 months and behaviorally after 3 and 6 months of repletion. Compared to controls, rats from low copper dams had lower brain copper and iron concentrations 3 and 6 months after repletion; other biochemical differences were not detected. Behavioral assessments after 6 months of repletion found a persistent impairment in motor function of rats from low copper dams, evaluated by the accelerating rotorod procedure. Impact: Findings indicate that impairment to motor function can persist following long-term recovery from perinatal copper deficiency.

Dietary zinc and in situ skeletal muscle function. This project was designed to examine the effects of low compared to adequate dietary zinc (4.5 vs 15 mg/kg) on lower leg muscle strength and fatigue in male rats. We used an in situ preparation and confirmed that marginal zinc retards peak force development (strength) and was associated with reduced time to muscle fatigue. Low zinc was related to decreased carbonic anhydrase activity in the muscles studied. Impact: These findings are consistent with the results of a previous pilot study and provide new evidence that low dietary zinc in an amount generally consumed by some groups of physically active people results in altered muscle function.

Mild iron deficiency affects mechanisms of temperature regulation. This study examined the effects of iron deficiency without anemia on body temperature and homeostasis. Rats were fed diets with graded dose amounts of iron (8, 15 and 45 mg/kg) then randomized to exposures of room temperature or cold air. Rats fed the low dietary iron had a small rate of loss of body temperature not different than the rates determined in the rats fed adequate or supplemental iron. Importantly, mitochondrial uncoupling protein, a key regulator of mitochondrial energy production, decreased as well as a specific heat shock protein, only in the rats fed the low iron diet. These findings suggest that marginal iron deficiency (e.g., non anemia) does not adversely affect temperature regulation in the cold. However, low iron does impair the capacity of mitochondria to produce heat as well as reduces the intracellular signals (heat shock proteins) that modulate catecholamine and thyroid hormone actions to promote heat generation. Impact: Whereas lack of anemia apparently has no measurable effect on body temperature, it does adversely mitochondrial heat production during acute cold exposure.

Dietary copper and markers of oxidative stress. Copper plays a key role in protection against oxidative stress through two specific copper-containing enzymes, superoxide dismutases. We evaluated the effects of feeding graded dose amounts of copper (0.5, 1, 1.5, 2, 2.5, and 6 mg/kg) on various biochemical measures of copper status in female and male growing rats during a 6-wk period. Iron status, shown by increased red cell width distribution, was affected by dietary copper with no evidence of anemia. Importantly, extracellular superoxide dismutase (SOD3) was a very specific marker in all rats. However, it was a sensitive indicator of dietary copper only in male rats; SOD3 activity was directly related to dietary copper. Among the female rats, SOD3 activity was reduced only in severe copper restriction.

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An equally important finding was that muscle cytochrome c oxidase, another copper-containing enzyme, was a sensitive and specific indicator of dietary copper in all rats and independent of muscle composition (e.g., fast- or slow-twitch). These findings indicate that these enzymes are novel markers of dietary copper. Impact: The identification and validation of these enzymes support the roles of copper in antioxidant protection and energy metabolism, and provides new opportunities for study of effects of copper restriction in metabolism.

C. Significant Accomplishments/Activities that Support Specific Target Populations. Mental and physical health, activity and nutrition in Northern Plains Indians. We compiled a preliminary report summarizing findings for each of five American Indian tribes participating in a two-year study to evaluate culturally appropriate research methods and to characterize basic relationships among mental and physical health, nutrition, physical activity, and social factors in Northern Plains Indians living on North Dakota's four reservations and one Minnesota reservation. We found that depression in NPI is strongly related to food insecurity and moderately related to less exercise, higher body mass index, and poorer physical health and diet, all moderated by gender. Impact: Findings per se and the sharing of them with tribal leaders and health care providers serve as a foundation for future studies to determine specific nutrient needs and the impact of diet and activity on health issues endemic to American Indian communities, with the goal of implementing effective, culturally appropriate, community-based interventions to improve health and quality of life. This study has also helped develop key relationships between regional American Indian communities and the USDA, critical to the success of future nutrition studies with this under-served population group.

D. Progress Report
Effects of dietary selenium source on cognition and brain electrophysiology. Collected brain electrophysiology, cognitive performance and mood states data and began analyses to determine the relative benefits for brain function and cognition of feeding selenium-enhanced meat, wheat and broccoli to adults in the Peoples Republic of China. Analyses continue under replacement project.

Effects of dietary copper and zinc intakes on cognition, brain and cardiovascular function. Data collection continued in a multi-year study to determine the individual and combined effects of dietary copper and zinc intakes on cognitive function, particularly memory, brain electrophysiology, and cardiovascular function of healthy postmenopausal women participating in an ongoing controlled metabolic unit study. Findings will help determine needed intakes of copper and zinc for optimal cognitive and brain function, and healthy cardiovascular function in older individuals, and will clarify previously observed interactive effects of copper and zinc on memory function of older women.

Dietary boron deprivation, sensory function and brain development. An experiment was performed to determine whether dietary boron deprivation impairs sensory functions and brain development, and whether any boron effect is altered by the lipid composition of the diet. The animal portion of the experiment has been completed in which dietary fat was either fish oil or safflower oil in boron-deficient or boron-adequate diets fed to rats. Analysis of behavioral measures of sensory function and brain development will be accomplished under the replacement project.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**
This bridging project was a consolidation of prior projects 5450-51000-027-00D,

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5450-51000-006-00D and 5450-51000-007-00D, and scheduled for 2 years (02/15/02-4/30/04). This project terminated 04/02/04 and was replaced by project 5450-51530-009-00D, Micronutrient Roles in Physiology and Health. This is the FINAL REPORT for the bridging project.

Zinc fortification improves memory of young adolescents. Completed data collection from the second and third cohorts of subjects in a multi-year field study of the relationship between zinc intakes and status, body composition, cognitive and adaptive function, and psychoeducational performance in 204 children, aged 12-14 years. Analysis of data from the first two cohorts showed that baseline plasma zinc was inconsistently but significantly related to several measures of attention in girls and to perception and reasoning in boys. Compared to placebo, 20 mg zinc fortification for 10 weeks improved memory overall (girls: 11% vs 6%; boys: 11% vs 8%) and short-term memory (girls: 13% vs 7%; boys: 11% vs 4%) in particular. Impact: Findings help determine needed zinc intakes to promote optimal physical and behavioral development of peri-pubescent adolescents at-risk for zinc deficiency because of rapid growth.

Abnormal motor function persists following recovery from perinatal copper deficiency. Pregnant rats were fed a low copper diet (0.34 mg/kg) starting on gestation day 7; half also received additional copper in their drinking water (20 mg/L) while the other half drank demineralized water. Pups from all dams were switched to a copper adequate diet (13.2 mg/kg) at weaning and sampled biochemically after 3 months and behaviorally after 3 and 6 months of repletion. Compared to controls, rats from low copper dams had lower brain copper and iron concentrations 3 and 6 months after repletion; other biochemical differences were not detected. Behavioral assessments after 6 months of repletion found a persistent impairment in motor function of rats from low copper dams, evaluated by the accelerating rotorod procedure. Impact: Findings indicate that impairment to motor function can persist following long-term recovery from perinatal copper deficiency.

Mental and physical health, activity and nutrition in Northern Plains Indians. We compiled a preliminary report summarizing findings for each of five American Indian tribes participating in a two-year study to evaluate culturally appropriate research methods and to characterize basic relationships among mental and physical health, nutrition, physical activity, and social factors in Northern Plains Indians living on North Dakota's four reservations and one Minnesota reservation. Study data were collected using a mobile research laboratory that traveled to 8 pow-wows and health fairs held on the reservations. We found that depression in NPI is strongly related to food insecurity and moderately related to less exercise, higher body mass index, and poorer physical health and diet, all moderated by gender. Impact: Findings per se and the sharing of them with tribal leaders and health care providers should serve as foundations for future studies to determine specific nutrient needs and the impact of diet and activity on health issues endemic to American Indian communities, with the goal of implementing effective, culturally appropriate, community-based interventions to improve health and quality of life. This study has also helped develop key relationships between regional American Indian communities and the USDA, critical to the success of future nutrition studies with this under-served population group.

Mineral nutrition and stress responses in women with dysmenorrhea. Previous studies in this and other laboratories have shown that mineral intakes affect menstrual symptomatology. We tested the hypotheses that calcium, magnesium and other mineral intakes and status mediate the stress responses (autonomic nervous system, corticosteroid concentration, prostaglandin production) of women with dysmenorrhea.

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We found that dietary intakes and status of both calcium and manganese status were lower among dysmenorrheic women, but did not affect responses to environmental or psychological stressors. Impact: Findings indicate that mineral nutrition is related to dysmenorrhea but future studies are needed to characterize their role.

Zinc deficiency and stress-related memory impairments. A study was conducted to determine in mature rats the interactive effects of increased glucocorticoid concentrations and restricted zinc intakes on hippocampal damage and working and reference memory, activity and emotionality. Previous studies found that environmental and other stressors increase glucocorticoid concentration in blood, damage cells in the hippocampal region of the brain and may impair memory. Independently, zinc deficiency has been linked to hippocampal damage, memory impairments and hyperemotionality. This study found that zinc deficiency did not increase risk of stress-related memory impairments and that adequate zinc intakes did not protect against such impairments. Impact: Despite affecting a common organ system, zinc deficiency and stress act independently on memory.

Regional Bioelectrical Impedance Analysis (BIA) devices fail to accurately measure body fat. The increased emphasis by national and international public health agencies has stimulated the development of devices for routine, personal assessment of body composition. We evaluated the validity of commercially-available bioelectrical impedance devices that use foot-to-foot or hand-to-hand electrode placements to measure body fatness and compared the results with dual x-ray absorptiometry in 62 women and 48 men, 20 to 72 years of age. Compared to the reference method, the regional impedance instruments significantly underestimated body fatness except the foot-to-foot placement in the men. Impact: These findings indicate that regional bioelectrical impedance devices that are marketed to the public are inaccurate because of an unequal distribution of muscle between the upper and lower body more so in females than males.

Dietary zinc restriction adversely affects muscle cell metabolism. Zinc is a limiting nutrient in the diets of adolescents, many of whom engage in physical activities to promote physical performance. A study was completed to determine the effects of dietary zinc restriction on activity and expression of carbonic anhydrase, a zinc-containing enzyme, in skeletal muscle in response to endurance exercise training. At a dietary level of zinc (4 mg/kg diet) consistent with that consumed by physically active individuals engaging in chronic endurance activities, carbonic anhydrase activity and protein concentrations in slow-twitch muscle were significantly decreased compared to rats fed a zinc-adequate (15 mg/kg) diet. This study also confirmed previous findings from our laboratory in which carbonic anhydrase isozyme activities in red blood cells were significantly decreased when dietary zinc was limiting (4.5 mg/kg). Impact: These findings show that dietary zinc adversely affects zinc-containing enzymes used to promote energy utilization and gas exchange between cells and the blood.

Reduced zinc status may impair muscle function. A pilot study was conducted to evaluate the effects of moderate zinc deficiency on in situ muscle function. Rats fed a diet containing 4.5 compared to 15 mg zinc per kg of diet had reduced force production and endurance during electrical stimulation of the isolated hind-limb muscles. Impact: These observations suggest a key role of zinc in regulating muscle function in vivo. Additional work is needed to improve technical aspects of the experimental procedure before final confirmation of the role of zinc in muscle function can be stated.

Dietary zinc and in situ skeletal muscle function. This project was designed to

examine the effects of low compared to adequate dietary zinc (4.5 vs 15 mg/kg) on lower leg muscle strength and fatigue in male rats. We used an in situ preparation and confirmed that marginal zinc retards peak force development (strength) and was associated with reduced time to muscle fatigue. Low zinc was related to decreased carbonic anhydrase activity in the muscles studied. Impact: These findings are consistent with the results of the previous pilot study and provide new evidence that low dietary zinc in an amount generally consumed by some groups of physically active people results in altered muscle function.

Sub-clinical iron deficiency alters mitochondrial signal transduction but does not impair thermoregulation in the cold. Iron deficiency anemia has been shown in humans to result in impaired thermoregulatory capacity during acute cold exposure. To determine the effects of iron deficiency with and without anemia, we fed rats diets containing a range of iron (5, 8,15 and 45 mg/kg) to induce severe and moderate iron deficiency compared to adequate iron status. The rats in each group were randomized to exposures of room temperature or cold air for a six-hr period. Rats fed the very low dietary iron became anemic and those fed the moderate iron diet did not; the other groups of rats had normal hemoglobin concentrations. The anemic rats experienced a faster rate of decrease in body temperature than the rats fed the moderate iron diet (0.08 degrees) which was not different that the rates measured in the rats fed adequate or supplemental iron. Importantly, mitochondrial uncoupling protein, a key regulator of mitochondrial heat production, decreased as well as a specific heat shock protein, only in the rats fed the very low iron diet. These findings suggest that only severe iron deficiency, evidenced by anemia, adversely affects temperature regulation in the cold by inhibiting the cellular mechanisms of thermogenesis. However, moderate dietary iron also reduced mitochondrial mechanisms of heat production. Impact: Whereas lack of anemia apparently has no measurable effect on body temperature, it does adversely mitochondrial heat production during acute cold exposure.

Dietary copper and markers of oxidative stress. Copper plays a key role in protection against oxidative stress through two specific copper-containing enzymes, superoxide dismutases. We evaluated the effects of dietary copper (0.5, 1, 1.5, 2, 2.5, and 6 mg/kg) on various biochemical measures of copper status in female and male growing rats during a 6-wk period. Iron status, shown by increased red cell width distribution, was affected by dietary copper with no evidence of anemia. Importantly, extracellular superoxide dismutase (SOD3) was a very specific marker in all rats. However, it was a sensitive indicator of dietary copper only in male rats; SOD3 activity was directly related to dietary copper. Among the female rats, SOD3 activity was reduced only in severe copper restriction. An equally important finding was that muscle cytochrome c oxidase, another copper-containing enzyme, was a sensitive and specific indicator of dietary copper in all rats and independent of muscle composition (e.g., fast- or slow-twitch). These findings indicate that these enzymes are novel markers of dietary copper. Impact: The identification and validation of these enzymes support the roles of copper in antioxidant protection and energy metabolism, and provides new opportunities for study of effects of copper restriction in metabolism.

New method for isolating mitochondria from brain and muscle. Methodology was developed to isolating viable, respiring mitochondria from brain and muscle by differential centrifugation. Development of methodology needed for quantifying protein carbonyls and nitrotyrosine in mirochondria and other cellular components was initiated.

Beneficial effects of boron and long-chain omega-3 fatty acids on eye development.

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Both boron and long-chain polyunsaturated fatty acids generated from alpha-linolenic acid (omega-3 fatty acids) have beneficial effects for many of the same life processes including eye development or function, apparently through affecting cellular membranes. Rats that were fed boron-deficient or -adequate diets containing either canola oil (high in omega-3 fatty acids) or palm oil (low in omega-3 fatty acids) through gestation and lactation produced pups that were fed the same diet for three weeks post-weaning. Examination of the eyes of pups by electron microscopic procedures revealed that a diet high in omega-3 fatty acids and boron improved mitochondrial configuration, condition, and abundance of cristae. The findings are consistent with an effect of dietary boron on ocular development/function.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
Transfer of technical information to other scientists occurred through presentations at national and international meetings and professional publications. Knowledge about the health benefits of mineral nutrients was transferred by routine contacts to representatives of industry and policy-making and regulatory federal agencies. Transfer of knowledge to the public occurred through contacts with media representatives and by direct contacts with the public.

Organized and facilitated discussion sessions at the Second American Indian Research Forum, held at the University of North Dakota, Grand Forks, ND, March 31, 2004. The forum provides researchers, community and Native leaders involved or interested in American Indian research an opportunity to discuss past, present and future research with Native peoples. Accomplished forum objectives to: (1) disseminate past research findings; (2) increase awareness of current and future research projects in the region; (3) forge new research collaborations and partnerships; (4) identify research priorities; and, (5) evaluate the effectiveness of culturally appropriate, community-based methods critical to success of research. Inadequate communication, cooperation and collaboration of individuals involved in research with American Indians has historically been a major barrier to enhancing the efficacy and value of research to Native peoples.

Contributed chapters "Boron" and "Silicon" to the document "Mineral Tolerance of Domestic Animals", prepared by the National Academy of Sciences, National Research Council, Committee on Minerals and Toxic Substances in Diets and Water for Animals,.

There are no CRADAs, licenses, or patents to report.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
Popular Press
Lukaski, H.C. Working Off the Holidays. Grand Forks Herald. November 10, 2003.

Lukaski, H.C. Working Off the Holidays. Government Employee Hospital Association Health e-Report. November 2003.

Nielsen, F.H. Increase Dining Pleasure with Essential Nutrients. Grand Forks Herald. December 3, 2003.

Gray, J.S. Vision Quest: American Indian Nutrition Research. Grand Forks Herald. January 7, 2004.

Project Number: 5450-51530-008-00D **Accession:** 0405210 **FY:** 2004

Presentations
Lukaski, H.C. Zinc and Copper - New Roles in Support of Physical Activity in Humans. Department of Nutrition and Food Science, University of Delaware, Newark, DE. October 3, 2003.

Lukaski, H.C. Supplements of Campus - Facts and Fantasy. Annual Meeting of the North Central College Health Association, Grand Forks, ND. October 17, 2003.

Lukaski, H.C. Fluid Status in Health and Disease. New Application of an Old Method: BIA Vector Analysis. Latin American Congress on Nutrition, Acapulco, Mexico. November 12, 2003.

Lukaski, H.C. Opportunities for Clinical Research in Federal Service. BioOpportunities Program, Interdepartmental Biological Sciences Program, Northwestern University, Evanston, IL. November 18, 2003.

Lukaski, H.C. Zinc and Copper - Regulators of Energy Efficiency During Exercise. Department of Preventive Medicine, Northwestern University School of Medicine, Chicago, IL. November 19, 2003.

Lukaski, H.C. "Low Carb" Diets in Weight Loss - Facts and Speculation. Wellness Program, Grand Forks Public Schools, Grand Forks, ND. January 12, 2004.

Lukaski, H.C. Small Steps to Increasing Physical Activity. USDA, ARS Grand Forks Human Nutrition Research Center, Grand Forks, ND. February 12, 2004.

Nielsen, F.H. Micronutrients and Animal Nutrition. International Fertilizer Association, New Delhi, India. February 2004.

Lukaski, H.C. Small Steps, Big Rewards to Attain and Maintain Healthful Body Weight. USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, March 17, 2004.

Lukaski, H.C. New Roles of Zinc and Copper in Promotion of Physical Performance in Humans. Tufts University Human Nutrition Research Center on Aging, Boston, MA, March 22, 2004.

Penland, J.G. American Indian Research Projects Approved by the University of North Dakota Institutional Review Board, 1990-2004. American Indian Research Form, University of North Dakota, Grand Forks, ND. March 31, 2004.

Scientific Publications: **Log 115:**

1. Droke, E.A., Briske-Anderson, M., Lukaski, H.C. Fatty acids alter monolayer integrity, paracellular transport, and iron uptake and transport in Caco-2 cells. Biological Trace Element Research 95:219-232, 2003. **0000130062**
2. Lukaski HC. Regional bioelectrical impedance analysis: Applications in health and medicine. Acta Diebetol 40:S196-S199, 2003. **0000147252**
3. Lukaski, H.C., Siders, W.A. Validity and accuracy of regional bioelectrical impedance devices to determine whole-body fatness. Nutrition, 19:851-857, 2003. **0000144845**
4. Nielsen, F.H. 2003. Trace elements. Book Chapter. In: Caballero, B., Trugo, L., Finglas, P., editors. Encyclopedia of Food Sciences and Nutrition. 2nd edition. London, England: Academic Press. p. 5820-5828. **0000110608**

Project Number: 5450-51530-008-00D **Accession:** 0405210 **FY:** 2004

5. Penland, J.G., Prohaska, J.R. 2004. Abnormal motor function persists following recovery from perinatal copper deficiency in rats. Journal of Nutrition. 134(8):1984-1988. **0000163703**

Approved: ROOS ERIC E **Date:** 09/14/2004

Project Number: 5450-51000-032-00D **Accession:** 0405026 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 02/23/2002 **Term Date:** 01/14/2004

National Programs: 107 100% Human Nutrition

Title: MINERAL UTILIZATION & BIOAVAILABILITY IN THE 21ST CENTURY, WITH CHANGING DIETS & AGRICULTURAL PRACTICE

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** Yes
Terminate in Two Months? No

Progress and Outcomes:

1. **What major problem or issue is being resolved and how are you resolving it (summarize project aims and objectives)? How serious is the problem? What does it matter?**

This is the annual and termination report for project 5450-51000-032-00D, which has been replaced by a related new OSQR-approved project, 5450-51000-035-00D that is being reported separately.
The nutritional and health status of the human population of the United States could be adversely affected by current and proposed changes in the composition of the diet. Such changes include 1) an emphasis on plant-based diets with limited intake of red meat, 2) nutrient fortification or supplementation of diets as proposed by health care professionals, or independently initiated by producers, and 3) production of genetically- or chemically-modified foods. This project has helped determine whether proposed changes to the US diet may affect the nutritional content of food; the absorption, bioavailability and metabolism of nutrients; and/or disrupt the balance of nutrients that could result in adverse nutrient/nutrient interactions.

Although many nutritional components were studied, the emphasis was on mineral nutrients. This included evaluation of influence of modified dietary selenium forms and concentrations for improving immune and neuropsychological function, antioxidant defense, and cancer risk in humans. Cellular, animal, and molecular biology methods were used to assess how the food forms of selenium affect its nutritional utilization and distribution into selenoproteins. The effect of common dietary variables on iron and zinc bioavailability and requirements were assessed in human and animal studies. Human studies assessed iron bioavailability from plant-based diets with reduced meat content, as influenced by control of heme and nonheme iron absorption, iron excretion, and human genotype. Human studies assessed the efficacy of heme iron supplements and of elemental iron fortificants for improving iron status. Further human studies tested the impact of high phytic acid content of or calcium fortification of foods on iron absorption. Human intestinal cell culture models and transgenic animal models were used to determine how newly identified transport proteins for iron, zinc, and copper regulate the absorption of and interactions between these nutrients. Cellular and animal studies evaluated the benefits of minor crops grown in the Northern Plains areas, such as buckwheat and flax, for improving mineral intake, altering lipid concentration, increasing antioxidant status, and decreasing the risk of cancer and diabetes.

This project is part of National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%). It is directly related to Human Nutrition Performance Goal 3.1.1 Human Nutrition Requirements and 3.1.3 Nutritious Plant and Animal Products, concerning the priority objective: Bioavailability of Nutrients and Food Components. It is in the same management unit and is coordinated with CRIS 5450 51000 031 00D, "Role of Dietary Minerals on Gene Expression, Cell Cycle and

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Molecular Mechanisms in Cancer Risk", especially in relation to Se containing food components with possible cancer preventive properties. It also coordinates with CRIS 5450-51000-034-00D, "Mineral Intakes for Optimal Bone and Joint Development and Health", in measuring calcium bioavailability from diets.

This research will be useful to food producers for the development and promotion of healthy foods, and to health care officials and educators for the development of dietary advice that contributes to optimal nutrition, including cancer prevention, reduction of iron deficiency anemia, reducing the retention of toxic minerals, and developing recommendations for dietary intakes and modifications. The ultimate beneficiary is the American consumer through policies and guidelines set by scientists and health care professionals based on these research findings and through transfer of enhanced agricultural products that improve nutrition worldwide.

2. **List the milestones (indicators of progress) from your Project Plan.**

Milestones (FY2003 was the first year that milestones were established for this combined interim CRIS project, for research that previously was included in several smaller CRIS projects)

Year 1 (FY2003)

Test a new experimental approach to determining zinc requirements in humans, by determining how people adapt their zinc absorption to different dietary zinc intakes.

Determine whether people with the common genetic mutation associated with the iron storage disorder, hemochromatosis, absorb more heme and nonheme iron than those without the mutation.

Determine how mineral nutrient status affects cadmium risk assessment in foods.

Determine how production conditions and cultivars affect the uptake of minerals by wheat and broccoli.

Year 2 (FY2004)

Determine the bioavailability to humans of two elemental iron powders commercially produced to fortify cereal grains, and test how the absorption of elemental electrolytic iron powder is affected by interactions with ingested ascorbic and phytic acids.

Determine the influence of micronization and encapsulation of ferric pyrophosphate, an iron form used in food fortification, on iron bioavailability to rats.

Begin studies too determine the basic mechanisms of how food zinc, iron, copper, and cadmium are absorbed from the gut (e.g., produce antibodies to specific transport proteins involved in iron, copper, and zinc uptake and transport; use the metallothionein knockout mouse to determine the relationship between intestinal metallothionein and cadmium accumulation in the duodenal enterocytes).

Begin basic studies to determine the mechanism by which selenium in meat, wheat and broccoli inhibit colon cancer. Conduct selenium supplementation studies in humans with inadequate and adequate selenium intake.

Compare conventionally-grown broccoli to organically-grown broccoli and determine whether production system has more of an impact on the nutritional profile than other environmental variables such as climate, water usage and season.

Characterize the phenolic acids in broccoli and determine whether they are associated with some of the anti-oxidant properties of broccoli.

(For future years, see report for new CRIS project 5450-51000-035-00D.)

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3. **Milestones:**

A. Milestones that were scheduled for FY2004:

All of the following scheduled milestones were completed:

Determine the bioavailability to humans of two elemental iron powders commercially produced to fortify cereal grains, and test how the absorption of elemental electrolytic iron powder is affected by interactions with ingested ascorbic and phytic acids. Both studies were completed and manuscripts are being prepared (see also sibling CRIS 5450-51000-035-13T).

Determine the influence of micronization and encapsulation of ferric pyrophosphate, an iron form used in food fortification, on iron bioavailability to rats. This animal study was completed, and further supportive in vitro work (particle size distribution, solubility) is in progress.

Begin basic studies to determine the mechanism by which selenium in meat, wheat and broccoli inhibit colon cancer. Conduct selenium supplementation studies in humans with inadequate and adequate selenium intake. Human studies are now in progress in Grand Forks and in China.

Compare conventionally-grown broccoli to organically-grown broccoli and determine whether production system has more of an impact on the nutritional profile than other environmental variables such as climate, water usage and season. Data was collected, analyzed and a manuscript is in preparation.

Characterize the phenolic acids in broccoli and determine whether they are associated with some of the anti-oxidant properties of broccoli. Data was collected, analyzed and a manuscript submitted.

The following scheduled milestone was completed, with the exception that, rather than produce antibodies to study mineral transport proteins, these antibodies are being obtained either commercially or privately from other investigators:

Begin studies to determine the basic mechanisms of how food zinc, iron, copper, and cadmium are absorbed from the gut (e.g., produce antibodies to specific transport proteins involved in iron, copper, and zinc uptake and transport; use the metallothionein knockout mouse to determine the relationship between intestinal metallothionein and cadmium accumulation in the duodenal enterocytes).

B. Milestones projected for FY2005, 2006, and 2007: See report for new CRIS project 5450-51000-035-00D.

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment during FY 2004:

a) Ferrous sulfate, reduced, electrolytic, and heme iron can all improve body iron status. The global problem of iron deficiency anemia emphasizes the need for effective and economical forms of iron for food fortification and supplementation. Ferrous sulfate is known to be well absorbed, but can cause adverse changes in food with storage, in contrast with elemental iron powders that may not be as well absorbed, and with hemoglobin, a well-absorbed form of iron of high molecular weight that would adversely color foods. With funding from USDA CREES Nutritional Research Initiative competitive grant 2002-01885 (tracked by CREES as CRIS 0192629 and by ARS as 5450-51000-035-13T), we examined the efficacy of fortifying food with elemental iron powders, relative to equivalent amounts (50 mg/d) of iron as ferrous sulfate, or of supplementing with a limited amount of iron (5 mg/d) in the heme form, on iron status in women with low iron stores. In a randomized, blinded, placebo-controlled efficacy trial, 52 premenopausal women with moderate to low iron stores received the heme iron in 2 capsules/d and the other sources in 3 wheat rolls/d for 12 wk. Body

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iron, assessed from the serum transferrin receptor/serum ferritin ratio increased significantly with all four iron sources, but not with placebo. The results indicate that the reduced and electrolytic iron sources were approximately 50 and 85% as effective as ferrous sulfate and that 5 mg iron in the heme form was half as effective as 50 mg of iron from ferrous sulfate for improving body iron in humans. These results can be useful in selecting forms and amounts of iron to use for food fortification.

B. Other significant accomplishments:

b) Not all micronized ferric pyrophosphate products are equivalent iron fortificants. New micronization techniques, accompanied with encapsulation treatments to maintain micronization, have been proposed to improve the solubility and resulting bioavailability of iron products such as ferric pyrophosphate. The bioavailability of ferric pyrophosphate sources with different commercial micronization and encapsulation treatments was tested by evaluating their efficacy in reversing iron deficiency anemia in rats, in research supported by PATH (Program for Appropriate Technology in Health). The results showed that one commercial product was nearly as bioavailable as ferrous sulfate, but that the other micronized and encapsulated products were no more bioavailable than ordinary ferric pyrophosphate. Further measurements of particle size distribution of these iron sources confirmed that the most bioavailable ferric pyrophosphate product in vivo had been micronized and encapsulated to a smaller mean particle size than the other products. This research provides information useful in determining economical and efficacious iron fortification sources to help combat the serious global problem of iron deficiency anemia.

c) An increased risk of cadmium accumulation with marginal intakes of zinc, iron, and calcium appears unrelated to intestinal metallothionein induction. Marginal intakes of the essential mineral nutrients zinc, iron, and calcium will enhance the absorption of cadmium from low-cadmium diets and may put the consumer at higher risk of cadmium exposure. Such intakes also stimulate the accumulation of cadmium in the upper small intestine (duodenum) of the rat model. Previous studies by others indicated that the intestinal metal accumulator, metallothionein (MT), located in the intestinal epithelial cells is up-regulated and accumulates cadmium. A study at the Grand Forks Human Nutrition Research Center tested cadmium accumulation as affected by marginal vs. adequate zinc, iron and calcium, in both control and mutant mice that do not contain a functional MT protein. When the mice were fed diets containing low cadmium and marginal levels of zinc, iron, and calcium, they accumulated 10 times more cadmium than controls fed low cadmium and adequate minerals. When fed similar diets, the mutant mice accumulated similar amounts of cadmium as the control mice with intact MT. The large increase in duodenal cadmium was not accompanied by an increase in MT concentration in the intestinal cells. These data suggest that MT induction is not a casual factor in intestinal cadmium accumulation and subsequent absorption in animal models fed low cadmium and diets marginally deficient in zinc, iron, and calcium. A better understanding of the mechanism of this nutritional interaction may be helpful in developing policies to guard against cadmium toxicity in exposed populations.

d) Copper deficiency reduces iron absorption in male rats. Copper deficiency causes iron deficiency anemia in most animal species. It was hypothesized that part of the problem was that copper deficiency inhibits iron absorption from the intestinal tract. However, previous research by others, using insensitive techniques, obtained mixed results - some found that iron absorption was reduced in male animals; some found that it was enhanced in female animals. To determine if indeed copper

deficiency reduces the intestinal absorption of iron, a study conducted at the Grand Forks Human Nutrition Research Center used a test diet labeled with radioactive iron and state-of-the-art whole-body-counting techniques. Copper deficiency reduced iron absorption by about 50% in male rats. These findings suggest that at least part of the cause of iron deficiency anemia in copper-deficient animals is a lack of efficient absorption of dietary iron. An understanding of the role of copper in iron absorption will be useful in setting recommendations for balanced dietary intakes and addressing the causes of nutritional anemias.

e) An organic farming method had limited influences on several nutritional characteristics of broccoli. Many proponents of organic agriculture claim that organically-produced food is superior nutritionally to that produced by conventional methods. However, most studies have had serious flaws in the experimental design, and recent reviews conclude that there is very little evidence that organic farming impacts nutritional value. A comprehensive study to address this question was conducted over two growing seasons in CA. Broccoli was produced in two locations (coastal CA and the Central Valley of CA). Multiple farms at both locations produced broccoli by either conventional techniques or by certified organic technique; additionally crops were irrigated with 80, 100 or 150% of the water requirement, and within water treatment, multiple fertilizer treatment regimens were used. Broccoli was harvested at maturity and analyzed for trace minerals, multiple individual glucosinolates, primary glucosinolate breakdown products, vitamin C and phenolic acids; data were analyzed by stepwise regression models and discriminate analysis. The farming method was a significant predictor of nutritional quality for only a few compounds, and then it was of only secondary or tertiary importance. Additionally, no clear trend was discernable as organic production increased some compounds and decreased others. Location was by far the most important factor, and fertilizer regimen also was quite important in influencing nutritional content. These data provide initial evidence that when an entire range of variables are considered, the farming system is of limited importance for determining nutritional quality.

f) Enhancing the selenium content of broccoli reduced other bioactive compounds. Functional foods are sold because a compound in the food targets a specific metabolic or disease state. However increasing evidence is demonstrating that bioactive compounds in plants vary greatly depending on production variables. Broccoli contains three major bioactive compounds: glucosinolates, phenolic acids, and under some conditions, selenium. We have demonstrated that enrichment of broccoli with selenium dramatically decreases total glucosinolate content and specifically decreases sulforaphane, the most active glucosinolate in broccoli. Selenium enrichment also changes the phenolic profile of broccoli, especially reducing the content of hydroxy-cinnamic acids. Water stress also causes similar changes, but not as dramatic as selenium fertilization. These data show the impact of production variables on the accumulation of 'functional' compounds; they also suggest that it may not be possible to simultaneously maximize all bioactive ingredients in a food, as enrichment with one compound may cause a concomitant decrease in another.

5. **Describe the major accomplishments over the life of the project, including their predicted or actual impact.**

This project has investigated copper, iron, selenium and zinc absorption and metabolism. Some highlights include:

- marginal intakes of zinc, iron, and calcium greatly enhanced the accumulation of cadmium in the upper small intestine, leading to a higher accumulation of the toxic

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metal in the liver and kidneys, which suggests that populations with these nutrient deficiencies are especially susceptible to cadmium toxicity.
- a novel role for selenium in the up-regulation of cell cycle related genes was discovered, that may lead to a better understanding of the essentiality of selenium as a nutrient and its involvement in cancer prevention.
- although Se-methyl selenocysteine was the primary form of selenium in broccoli, methyl selenol was the primary form of Se absorbed by intestinal cells in culture, which may help explain the observed high efficacy of Se from broccoli for preventing colon cancer.
- elemental iron powders commonly used to fortify staple foods with iron were only 21-64% as bioavailable to rats as iron from ferrous sulfate, suggesting that higher concentrations of these forms may be needed if they are used in international iron fortification programs.
- in a sample of 262 healthy premenopausal US women, body iron stores were primarily predicted by a self-estimation of menstrual losses, and less substantially related to the dietary consumption of meat, phytic acid (from whole grains, legumes, and nuts), tea, or iron supplements, suggesting that low iron stores in many US women may be primarily related to increased menstrual loss, rather than poorer diets.
- copper deficiency reduced iron absorption in the rat experimental model by 50%, which in turn, leads to iron deficiency anemia. If similar effects are found in humans, they will strongly suggest that iron deficiency anemia as a world health problem should not be studied without knowledge of the nutritional copper status of the population affected. Iron supplementation for iron deficiency signs may be of no benefit without a parallel supplement of copper.

6. **What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end-user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products?**
J. R. Hunt served as a member of the National Academy of Sciences, Institute of Medicine, Standing Subcommittee on Interpretation and Use of Dietary Reference Intakes, July 1, 2000 - December 31, 2002, contributing to the report "Dietary Reference Intakes: Applications in Dietary Planning", Washington DC, National Academies Press, 2003, which can serve as a reference for scientists and policy-makers involved in dietary planning.

7. **List your most important publications in the popular press and presentations to organizations and articles written about your work.**
None

Scientific Publications: **Log 115:**

1. Reeves, P.G., Ralston, N.V., Idso, J.P., Lukaski, H.C. 2004. Contrasting and cooperative effects of copper and iron deficiencies in male rats fed different concentrations of manganese and different sources of sulfur amino acids in an AIN-93-G-based diet. Journal of Nutrition. 134:416-425. **0000153845**
2. Reeves, P.G. 2004. Definition of terms for the encyclopedia of farm animal nutrition. Book Chapter. In: Fuller, M.F., editor. The Encyclopedia of Farm Animal Nutrition. Oxon, UK:CABI Publishing, CAB International. p. variable. **0000113172**
3. Finley, J.W. 2004. Does environmental exposure to manganese pose a health risk to healthy adults? Nutrition Reviews. 62(4):148-153. **0000157210**
4. Hunt, J.R. 2003. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. American Journal of Clinical Nutrition. 78(Suppl):633S-639S. **0000136096**
5. Hunt, J.R. 2003. Two women who contributed to early vitamin and mineral **0000150548**

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research: Mary Swartz Rose and Helen T. Parsons. Journal of Nutrition. 133:3686-3689.

6. Hunt, J.R. 2003. High-, but not low-bioavailability diets enable substantial control of women's iron absorption in relation to body iron stores, with minimal adaptation within several weeks. American Journal of Clinical Nutrition. 78:1168-77. 0000149021

7. Reeves, P.G., Chaney, R.L. 2003. Bioavailability of food cd as an issue in risk management and assessment. Meeting Abstract. Scientific Committee on Problems of the Environment (SCOPE) Second Workshop on Risk Assessment and Management of Environmental Cadmium. Ghent, Belgium. Spetember 3-6, 2003. 0000153840

8. Chaney, R.L., Reeves, P.G., Ryan, J.A., Simmons, R.W., Welch, R.M. 2003. An improved understanding of soil Cd risk to humans [abstract]. Proceedings of the International Symposium on Health Impacts of Cadmium Exposure in China. p. 49-51. 0000155954

9. Chaney, R.L., Ryan, J.A., Reeves, P.G. 2003. Cadmium risk perception and assessment-principles and procedures. [abstract]. Abstracts of the SCOPE Workshop on Risk Assessment and Management of Environmental Cadmium. 0000153759

10. Finley, J.W., Hintze, K.J., Robbins, R.J., Keck, A. 2004. Activation of antioxidant responsive element (are) -regulated genes by bioactive components in broccoli - effects of fertilization with selenium (se) [abstract]. The Federation of American Societies for Experimental Biology Journal. 18:A892. 0000156320

11. Hunt, J.R., Zeng, H. 2004. Heme and nonheme iron absorption in humans heterozygous for the C282Y HFE mutation associated with hemochromatosis. Journal of Federation of American Societies for Experimental Biology. 18:A766. 0000156258

Approved: ROOS ERIC E **Date:** 09/14/2004

Project Number: 5450-51000-035-07G **Accession:** 0404348 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 01/01/2001 **Term Date:** 09/15/2004

National Programs: 107 100% Human Nutrition

Title: CHEMICAL FORMS OF SELENIUM IN FOODS

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 59-5450-1-0311

Organization Name: UNIV OF NORTH DAKOTA

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2002:
Speciation of the chemical form of selenium in plants is essential to helping explain the metabolism of selenium from those sources. Dr. T. Borgerding, in association with Dr. M. Roberge helped adapt an HPLC-inductively coupled plasma mass spectrometry method to our laboratory for the purpose of speciating high-selenium plants. The methodology was successfully transferred to USDA full-time employees (R. Sims and C. Lachler). Selenium enriched vegetables and wheat were successfully analyzed by this method. The successful transfer of this methodology to our laboratory will enable us to now speciate all plant material used in cell culture, animal and human selenium experiments.

B. Other Accomplishments.
Expired gases, especially methane and hydrogen, are indicators of fermentation in the lower bowel. As part of a human high-selenium wheat feeding experiment, subjects were fed either whole wheat or refined wheat and measures of fermentation were assessed. Drs. Bogerding and Roberge helped adapt a gas chromatography (GC) to our laboratory for the purpose of determining the concentration of expired methane and hydrogen in the breadth of these subjects. All analyses are complete and the data are being analyzed.

C. Special populations:
None

D. Progress Report:

This report serves to document research conducted under 59-5450-1-0311 Grant Agreement between ARS and the Department of Chemistry, University of North Dakota. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

The purpose of the project is to determine the chemical forms of Se in the high-Se foods that are being used in related projects. We also are attempting to determine the metabolism of Se compounds consumed in the diets of animals and humans.

Scientific Publications: **Log 115:**

Project Number: 5450-51000-035-07G **Accession:** 0404348 **FY:** 2004

1. Roberge, M.T., Finley, J.W., Lukaski, H.C., Borgerding, A.J. 2004. Evaluation of the pulsed discharge helium ionization detector for the analysis of hydrogen and methane in breath. Journal of Chromatography A. 1027:19-23. **0000151817**

Approved: ROOS ERIC E **Date:** 07/28/2004

Project Number: 5450-51000-035-08S **Accession:** 0404351 **FY:** 2004
ModeCode: 5450-20-00 NORTHERN PLAINS AREA
GRAND FORKS HUMAN NUTRITION RESEARCH CENTER
MICRONUTRIENT ABSORPTION AND METABOLISM
NPL Leader: KATHLEEN C ELLWOOD
Start Date: 01/01/2001 **Term Date:** 09/15/2004

National Programs: 107 100% Human Nutrition

Title: HEALTH BENEFITS OF HIGH-SELENIUM FOODS TO HUMANS

Period Covered **From:** 10/2003 **To:** 9/2004 **Final Report?** Yes
Terminate in Two Months? No

Agreement Number: 58-5450-1-0315

Organization Name: OREGON STATE UNIVERSITY

Progress and Outcomes:

4. **What were the most significant accomplishments this past year?**

A. Single most significant accomplishment of 2004:
The distribution of selenium between proteins found in blood is an indication of how Se is metabolically partitioned in the body. Selenium is primarily found in three proteins: serum albumin, and the selenoproteins plasma glutathione peroxidase and plasma selenoprotein P. The laboratory of Dr. P. Whanger has developed a gel filtration method to separate these proteins. Three high-selenium foods (high-selenium beef, wheat or broccoli) were used to provide supplemental selenium to subjects with extremely low selenium status (residents of DaChang China). Blood samples were taken at regular intervals, plasma proteins were separated and the concentration of selenium in each protein fraction is being determined. Data from these analyses will be valuable in interpreting other findings from human selenium feeding studies. Results will ultimately be utilized to determine which food form of selenium is the most bioavailable.

B. Other Accomplishments:
None

C. Special populations:
None

D. Progress Report:

This report serves to document research conducted under 58-5450-1-0315 Specific Cooperative Agreement between ARS and the Department of Environmental and Molecular Toxicology, Oregon State University. Additional details of the research can be found in the report for the parent CRIS 5450-51000-035-00D.

Dr. Whanger has a long-standing interest in Se metabolism, and especially how different forms of Se affect the use and distribution of Se in the body. Dr. Whanger was a co-author of the IFAFS grant award that is being used to pay this Specific Cooperative Agreement and Dr. Whanger has been instrumental in putting together the research agreement with the Chinese Academy of Preventive Medicine (#5450-51000-035-11S).

Publications:

Project Number: 5450-51000-035-08S **Accession:** 0404351 **FY:** 2004

None

Scientific Publications: **Log 115:**

Approved: ROOS ERIC E **Date:** 07/28/2004